PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 : C12Q 1/68 | A2

(11) International Publication Number: WO 00/66781

(43) International Publication Date: 9 November 2000 (09.11.00)

(21) International Application Number: PCT/US00/11577

(22) International Filing Date: 28 April 2000 (28.04.00)

(30) Priority Data:
60/131,732 30 April 1999 (30.04.99) US

(71) Applicant: BALL SEMICONDUCTOR, INC. [US/US]; 415 Century Parkway, Allen, TX 75013 (US).

(72) Inventors: ISHIKAWA, Akira; 846 FM 2453, Royce City, TX 75189 (US). TAKEDA, Nabuo; 2-38-7, Nishinodaira, Taihaku-ku, Sendai-si, Miyagi-ken 982-0825 (JP). AHN, Suzanne, I.; 7918 Glen Albens Circle, Dallas, TX 75225 (US). AHN, Samuel, S.; 256 South Beverly Glen, Los Angeles, CA 90024 (US). HAYS, Steven, R.; 7918 Glen Albens Circle, Dallas, TX 75225 (US).

(74) Agent: HOWISON, Gregory, M.; Howison, Chauza, Handley & Arnott, L.L.P., P.O. Box 741715, Dallas, TX 75374-1715 (US).

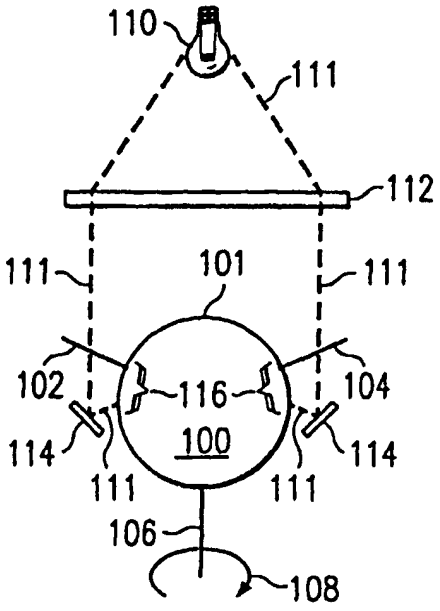
(81) Designated States: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
Without international search report and to be republished upon receipt of that report.

(54) Title: DNA BALLS

(57) Abstract

A rapid gene analysis architecture using a miniature spherical-shaped "ball" semiconductor device (100). In one aspect, an electron transfer detector (800) is implemented having each molecular probe (802) attached to a gate (810) of a multi-gated FET which is fabricated on the ball (100). An electron acceptor complex (804) is attached near the distal end of a short spacer chain (806) composed of poly(ethylene oxide), linking the molecular probe (802) to the ball (100). Multiple different molecular probes (802) each containing an electron acceptor complex (804) are placed on the ball (100). Target molecules (801) containing electron donor complexes (808) are passed over the ball surface (101) allowing hybridization to occur. Following hybridization, the ball surface (101) is then illuminated causing stimulation of the electron transfer from the donor complex (808) to the acceptor complex (804) located proximally to the gate (810) of the multi-gated FET. The electron transfer reaction is detected by the FET at a site where hybridization has occurred producing an electron transfer reaction. Conversely, if there are probe-target mismatches, the observed electron transfer does not occur. A signal will only be generated for those sites where hybridization is complete. Electron transfer occurs simultaneously at all locations where there was complete hybridization. Hybridization information is then transmitted from the ball (100) to a computer for processing. Hybridization information obtained from analyte-containing fluids passing over single balls or multiple ball addressable arrays in packed columns is transmitted to a computer for analysis and/or process control.



FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	**ES**	Spain	**LS**	Lesotho	**SI**	Slovenia
AM	Armenia	**FI**	Finland	**LT**	Lithuania	**SK**	Slovakia
AT	Austria	**FR**	France	**LU**	Luxembourg	**SN**	Senegal
AU	Australia	**GA**	Gabon	**LV**	Latvia	**SZ**	Swaziland
AZ	Azerbaijan	**GB**	United Kingdom	**MC**	Monaco	**TD**	Chad
BA	Bosnia and Herzegovina	**GE**	Georgia	**MD**	Republic of Moldova	**TG**	Togo
BB	Barbados	**GH**	Ghana	**MG**	Madagascar	**TJ**	Tajikistan
BE	Belgium	**GN**	Guinea	**MK**	The former Yugoslav Republic of Macedonia	**TM**	Turkmenistan
BF	Burkina Faso	**GR**	Greece			**TR**	Turkey
BG	Bulgaria	**HU**	Hungary	**ML**	Mali	**TT**	Trinidad and Tobago
BJ	Benin	**IE**	Ireland	**MN**	Mongolia	**UA**	Ukraine
BR	Brazil	**IL**	Israel	**MR**	Mauritania	**UG**	Uganda
BY	Belarus	**IS**	Iceland	**MW**	Malawi	**US**	United States of America
CA	Canada	**IT**	Italy	**MX**	Mexico	**UZ**	Uzbekistan
CF	Central African Republic	**JP**	Japan	**NE**	Niger	**VN**	Viet Nam
CG	Congo	**KE**	Kenya	**NL**	Netherlands	**YU**	Yugoslavia
CH	Switzerland	**KG**	Kyrgyzstan	**NO**	Norway	**ZW**	Zimbabwe
CI	Côte d'Ivoire	**KP**	Democratic People's Republic of Korea	**NZ**	New Zealand		
CM	Cameroon			**PL**	Poland		
CN	China	**KR**	Republic of Korea	**PT**	Portugal		
CU	Cuba	**KZ**	Kazakstan	**RO**	Romania		
CZ	Czech Republic	**LC**	Saint Lucia	**RU**	Russian Federation		
DE	Germany	**LI**	Liechtenstein	**SD**	Sudan		
DK	Denmark	**LK**	Sri Lanka	**SE**	Sweden		
EE	Estonia	**LR**	Liberia	**SG**	Singapore		

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

DNA BALLS

TECHNICAL FIELD OF THE INVENTION

This invention is related to the detection of molecules, inclusive of DNA, RNA and other proteins.

BACKGROUND OF THE INVENTION

The detection of molecular binding activity is a method of analysis which has a wide range of applications. The most widely publicized example of this is utilizing single-stranded deoxyribonucleic acid (ssDNA), as well as RNA, will hybridize with another ssDNA molecule attached to a surface, opened the door to an extremely powerful tool. The level of binding is then measured through the means of a detection scheme. Note that though this invention encompasses the use of DNA and RNA molecules, it is extended to include the use of any molecular binding agent for qualitative or quantitative detection of an analyte and process control involving said analyte.

People began attaching ssDNA and oligonucleotides (a single-stranded DNA or RNA molecule) to surfaces for simultaneous detection of various genes extracted from any tissue or cell culture of interest. A number of companies have developed means of coupling these oligonucleotides directly to glass or other media, including direct coupling to semiconductor silicon wafers to increase both the number of samples analyzed and to speed up the analysis through computer software integration. These oligonucleotide-bound silicon wafers have been termed "gene chips."

The disclosed embodiments use similar chemical procedures to synthesize oligonucleotides on a solid surface, notably a spherical semiconductor. The chemical synthesis for making very high-density arrays of oligonucleotides is described in a number of papers in the open scientific literature, for example, Livache et al., ANALYTICAL BIOCHEMISTRY 255(2):188-194 (1998), Hacia et al., NATURE GENETICS 14:441-447 (1996), and contained in a number of U.S. patents including: U.S. Patent No. 5,770,722 by Lockhart et al., and entitled "Surface-Bound, Unimolecular, Double-Stranded DNA;" U.S. Patent No. 5,763,599 by Pfleiderer et al., and entitled "Nucleoside Derivatives With Photolabile Protective Groups;" U.S. Patent No. 5,770,456 by Holmes, and entitled "Cyclic Nucleic Acid And Polypeptide Arrays;" U.S. Patent No. 5,753,788 by Fodor et al., and entitled "Photolabile Nucleoside Protecting Groups;" and U.S. Patent No. 5,837,832 by Chee et al., entitled "Arrays Of Nucleic Acid Probes On Biological Chips." The disclosed embodiments also cover the printing of

longer chain cDNA, PCR products or other purified strands (0.6 to 2.4 kb preferred size) to surfaces, as described by Duggan et al., in NATURE GENETICS SUPPLEMENT 21:10-14 (1999).

These surface-attached DNA fragments have been used for a number of purposes such as gene expression, (Duggan et al., NATURE GENETICS SUPPLEMENT 21:10-14 (1999)), DNA sequencing, detecting genetic mutations, genetic screening, and pathogen detection and analysis (Genetikai et al., ORV. HETIL. 139(16):957-60 (1998)), hepatitis C virus genotyping from blood samples (Livache et al., ANALYTICAL BIOCHEMISTRY 255(2):188-194 (1998)), evolutionary sequence comparisons between species (NATURE GENETICS 18(2):155-8 (1998)), detection of heterozygous mutations in BRCA1, and the hereditary breast and ovarian cancer gene (Hacia et al., NATURE GENETICS 14:441-447 (1996)). In the most typical format, the sample is prepared by attaching a fluorescent tag to the DNA or RNA chain, or synthesizing a chain that already has fluorescent labels attached thereto using PCR techniques. In this way, hybridization is detected by the strength of the fluorescent signal at each specific location on the substrate. The substrate is then typically taken to a separate device, and the fluorescence is read and sent to a computer for analysis.

The basis of all human disease can at some level be traced to the information contained in the genetic code. This code is physically made up of billions of DNA monomers linked together in a very specific sequence. It is the sequence of these monomers that contains the information required for every organism to live, feed, reproduce and carry out all life functions. The human genome, the collection of all DNA molecules within the species, is made up of some 3.5 billion base pairs, that contain an estimated 100,000 genes (genetic code). Genes are specific segments of the DNA molecules that serve as the code for the fabrication of proteins. During reproduction, replication of the 3.5 billion base pairs can occasionally lead to deletions, insertions, or mistranslations of the genetic code. If such a change occurs within a gene, the resulting protein may have a different three-dimensional shape and potentially loose function. Occasionally this kind of change can lead to life threatening conditions such as, for example, cystic fibrosis, Huntington's chorea, and sickle cell anemia.

The clinic of the future may be able to sequence, and analyze each patient's total DNA

content, including DNA from invading organisms such as bacteria and viruses. Not only is detecting the presence of a specific bacteria or virus important, but analyzing for mutations within the bacteria's DNA may determine which medication will be more effective in treatment. In certain instances, medications can be designed or constructed specifically for the mutated bacteria or virus. This methodology requires analysis of very large amounts of data very rapidly. The "gene chip" has been developed as a first step to rapid, high-volume DNA analysis that directly feeds information to a computer system. The disclosed architecture expands the capabilities of such techniques. It likewise extends them to batch or continuous control of processes involving the analytes in clinical, agricultural, or other industrial applications.

The assignee of the present application has one or more pending applications and issued patents describing spherical-shaped semiconductor devices, or "ball" semiconductors, that can be advantageously adapted to facilitate practical embodiments of the disclosed novel aspects; U.S. Patent No. 5,955,776, issued September 21, 1999, entitled "Spherical-Shaped Semiconductor Integrated Circuit," and U.S. Patent Application Serial No. 09/448,642, filed November 24, 1999, entitled "Miniature Spherical-Shaped Semiconductor With Transducer," each of which is incorporated herein by reference.

SUMMARY OF THE INVENTION

The present invention disclosed and claimed herein, in one aspect thereof, comprises a method of rapid gene analysis. One or more molecule-receptive surface regions are provided on the surface of a spherical-shaped semiconductor device. The semiconductor device is then exposed to a medium containing molecules from a controlled source. The molecules are caused to bond to the one or more molecule -receptive surface regions of the semiconductor device. Bonding information is then sensed when the molecules bond at each of the one or more molecule -receptive regions. In another aspect, an array of spherical semiconductor devices is constructed by conventional interconnects, suitable for column chromatographic applications. The bonding information from single devices, or multiple device arrays is then transmitted by direct or RF electronic techniques to a computer for processing. Batch or continuous flow mass transfer processes involving the analytes is carried out with suitable actuators.

BRIEF DESCRIPTION OF THE DRAWINGS

For a more complete understanding of the present invention and the advantages thereof, reference is now made to the following description taken in conjunction with the accompanying Drawings in which:

FIGURE 1 illustrates binding of molecules to the surface of a ball semiconductor using mirrors and photochemistry;

FIGURE 2 is a schematic of the initial placement of binding molecules upon the ball surface;

FIGURE 3 shows schematically the addition steps for placement of additional monomers;

FIGURE 4 shows a gene ball with a molecule attached to the surface by a spacer of oligo(ethylene oxide);

FIGURE 5 shows a gene ball with an aminosilane surface coating for the binding of molecular chains;

FIGURE 6 illustrates molecules coupled to the surface of a gene ball using photo-reactive spacer molecules;

FIGURE 7 illustrates the ball fluorescence detector having photoemitter/detector pairs emplaced in recessed wells of the ball surface;

FIGURE 8 illustrates an electron transfer detector comprising electron donors and acceptors used to detect electron transfer from the complementary binding of a molecule to the surface probe;

FIGURE 9 illustrates a multi-gated FET semiconductor structure for the detection of electron transfer on a gene ball;

FIGURE 10 illustrates a liquid chromatography column packed with beads;

FIGURE 11 illustrates a general block diagram of the circuit embodiments of the molecular detection ball and an external control station;

FIGURE 12 illustrates a general schematic diagram of the circuit embodiments of the molecular detection ball and the external control station of FIGURE 11;

FIGURES 13A-C illustrate alternative embodiments for the transmit/receive operation;

FIGURE 14 illustrates a physical diagram of a molecular detection ball and associated exposed circuit blocks;

FIGURE 14A illustrates an addressable array of spherical semiconductors, tightly packed so as to permit column chromatographic processing of a fluid containing one or multiple analytes;

FIGURE 15 illustrates a cross section of a molecular detection ball;

FIGURE 16 illustrates a side view of an alternative embodiment utilizing additional circuitry or structure attached to the ball for providing a local power source;

FIGURE 17 illustrates a side elevation of a cluster or aggregate of semiconductor gene balls that may be employed in a sensor function, according to a disclosed embodiment;

FIGURE 17A illustrates a process control scheme, in which a sample of analyte-containing fluid is directed to a spherical semiconductor molecular detector, or packed array of detectors, wherein molecular species, time and position information is obtained and transmitted to an actuator, in this case an electronically controlled, multiple port fluid valve;

FIGURE 18 illustrates a cross section taken along the line 18-18 of FIGURE 17 to expose the four contacts between adjacent balls;

FIGURE 19 illustrates a cluster or aggregation of balls;

FIGURE 20 illustrates a more detailed semiconductor structure of the emitter/detector pairs of the fluorescence sensor; and

FIGURE 21 illustrates a conventional circuit block diagram of the photo emitter/detector circuits as fabricated and illustrated in FIGURE 20.

DETAILED DESCRIPTION OF THE INVENTION

Referring now to FIGURE 1, there is illustrated a ball 100 which is held by three support pins 102, 104, and 106, and has free rotational capabilities 108 in either direction about the z-axis which aligns along the longitudinal length of pin 106. Photo-chemistry is used to build molecular structures such as oligonucleotides on the surface using a series of photo-activated monomers (i.e., nucleosides). A light source 110 emits light 111 onto the ball 100 through selected areas of a mask 112. Light 111 is directed through the mask 112 to specific regions 116 on the surface 101 by a series of the mirrors 114. The mirrors 114 are then operable to reflect an adequate quantity of light received through the unmasked area of the mask 112 to the regions 116 which would otherwise not receive the necessary amount of light to facilitate the process. Light 111 having a designated wavelength is emitted from the light source 110 striking a molecular target complex on the surface 101 of the ball 100.

Referring now to FIGURE 2, there is illustrated a spacer molecule 200 which is first attached to the surface 101 of the ball 100. Most preferably, the spacer (or linker) molecule 200 is a hetero-bifunctional oligomer of ethylene oxide, typically between approximately 3 and 100 repeat units in length. The functional group at the proximal end 202 of the spacer molecule 200 allows coupling to the surface 101 of the ball 100, and the distal end 206 has the first reactive group 208 attached to it. The nature of the first reactive group 208 depends on the type of detector (or sensor) to be used. If the sensor is based upon electron transfer or electrochemical sensing, the first reactive group 208 has an electron acceptor attached to the surface, preferably Rh(phi) sub 2 phen sup 3 plus (phi, phenanthrenequinone diimine), as described by Murphy in SCIENCE 262(5136):1025-9 (1993). Regardless of the sensor type, the first reactive group 208 comprises a molecular target complex containing a photo-sensitive blocking group 212 linked to an activated ligand 210 via a photo-labile bond. (Note that a ligand is an agent which is recognized or bound by a particular receptor.) Light 111 is directed through the mask 112 to the specific regions 116 on the surface 101 by the series of mirrors 114. The light 111 of the designated wavelength is emitted from the light source 110 striking the molecular target complex breaking the photo-labile bond between the activated ligand 210 and the photo-sensitive blocking group 212. The activated ligand 210 is then available to bind

additional nucleosides.

Referring now to FIGURE 3, there is illustrated a ball 100 containing exposed, activated ligands 210 after the light source 110 has been turned off. In the event that an oligonucleotide is being constructed, a nucleoside (A, T, G, C) complex 300 containing a 3′ end coupler 302 (specifically designed to bind to the surface-bound activated ligand 210), and a 5′ end containing a photo-sensitive blocking end group 304, is then passed across the ball surface 101. This nucleoside complex 300 covalently binds exclusively in those locations where the light source 110 has activated the spacer chain 200, as illustrated in FIGURE 2. Nucleoside complexes 300, which do not react with activated ligand 210 sites, are washed from the surface 101. The process is then repeated using a different mask 112 and/or mirror 114 arrangement and the next pattern of light 111 shines on the surface 101, removing the nucleoside 5′ photo-sensitive blocking groups 304 at the same or other locations. A nucleoside complex 306 containing a 5′ photo-sensitive blocking group 304 and a "free" 3′ terminal 308 is then passed across the surface 101 allowing the free 3′ terminal 308 of the nucleoside complex 306 to bind to a surface-attached "free" 5′ terminal 310 exposed by the removal of the photo-sensitive blocking group 304.

Referring now to FIGURE 4, there is illustrated the process which is repeated until oligonucleotides 400 of the desired length are achieved on the surface 101 of the ball 100. The oligonucleotide length is typically between 8 and 30 nucleotides, with a count of 20 being the most common. By this method, 4^N combinations can be synthesized in only 4 x N steps. These oligonucleotides 400 are synthesized either directly on, or immediately adjacent to the sensors on the surface 101 of the ball 100. The number of different oligonucleotides 400 on the ball 100 is restricted only by the packing density of the sensors. This process could similarly be employed to fabricate other molecular probes of protein or polymeric nature.

Referring now to FIGURE 5, there is illustrated a micro-array format wherein the semiconductor ball 100 is coated with a mixture of methyltrimethoxysilane 500 and 3-aminopropyltriethoxysilane 502 using vapor phase chemistry, a process known to those familiar with the art. The balls 100 are cleaned and prepared for coating with one or more

solutions, for example, piranha solution (4:1 mixture of concentrated sulfuric acid and 30% hydrogen peroxide) or SC1 (NH_4OH:H_2O_2 in water) or SC2 (HCl:H_2O_2 in water), and may be followed by a dilute HF solution to remove all surface contaminants and expose surface silanol (Si-OH) groups 504 (the Si portion being, in this particular embodiment, the substrate semiconductor material of the ball 100). The balls 100 are then washed with water to remove excess acid solution, then chemically dried using washes with progressively dehydrating solvents, for example, isopropyl alcohol or acetone followed by methylene chloride.

The silane monomers (500 and 502) each have three reactive groups, one to bind to the surface silanol 504, and two to cross-link with other surface-bound monomers to form polymer networks of the silane 506 covalently attached to the surface of the ball semiconductor 100. This polymer coating is ultra-thin and homogeneous, readily controllable from one to eight molecule layers thick. The silane polymer network 506 is composed of a random arrangement of methylated and aminated side chains. The monomers are used in a ratio to give a surface concentration of amine groups of approximately 0.1 pmol per square millimeter as recommended by Southern et al. in NATURE GENETICS SUPPLEMENT 21:5-9 (1999). Once this is complete, the surface is prepared for attaching purified cDNA directly to the surface. This can be accomplished in at least two different ways. In one method, the binding molecule is printed directly onto the surface using micro-printing techniques. For example, technology from the ink-jet printing industry now allows routinely the printing of dots on surfaces as small as 5 mm in diameter with extremely high accuracy and precision. Using this micro-printing technology allows the placement of over 1500 individual spots of binding molecule on a single substantially spherical ball semiconductor having an approximate diameter of one millimeter.

Referring now to FIGURE 6, there is illustrated another embodiment in which a layer of photo-inducible oligomers of poly(ethylene oxide) 602 is printed onto the silane polymer network 600 (of NH_2 and CH_3) covering the surface 101 of the semiconductor ball 100. Purified molecular strands 604 can then be micro-printed over the layer of photo-inducible poly(ethylene oxide) oligomers 602. In either case, light is then used to cross-link the molecular strands 604 to the surface 101 of the ball 100. These molecular strands 604 are printed on the surface 101 in precise locations corresponding to the locations of the oligomers

602 on the ball 100. This allows each oligomer 602 to detect hybridization of a single immobilized molecular strand 604.

SENSOR EMBODIMENTS

There are three type of sensors disclosed for implementation with the semiconductor ball 100. The first sensor discussed hereinbelow, is the fluorescence sensor which uses one or more photo-emitter/detector pairs to energize and measure the photo response of a fluorophore. The second sensor is an electron transfer detector used in conjunction with an external light to facilitate hybridization. Illumination of the ball surface 101 stimulates transfer of electrons from a donor complex to an acceptor complex, the electron activity being detected as the electrons pass into the gate of a multi-gated field effect transistor (FET).

FLUORESCENCE SENSOR

Referring now to FIGURE 7, there is illustrated the ball fluorescence detector 700 having photosensors 702 emplaced in recessed wells 704 of the ball surface 101. The fluorescence sensor (or detector) 700 contains one or more light emitting diodes (LEDs) 701 that emit light at the excitation frequency for the one or more fluorophores of interest. (Fluorescence is emitted when a fluorophore interacts with an incident photon (excitation). Absorption of the photon causes an electron in the fluorophore to rise from its ground state to a higher energy level. Then, the electron reverts to its original level, releasing a photon (fluorescence emission) whose wavelength depends upon the amount of energy that is released during reversion. A given fluorophore may emit at single or multiple wavelengths (creating an emission spectrum) as electrons drop from various orbitals to their ground states. The emission spectrum is constant for each species of fluorophore.) Located proximate to the LEDs 701 are photo-detectors 702 that are programmed to detect light at the emission frequency of the given fluorophore. The photo-detectors 702 are recessed into the ball surface 101 within respective narrow wells 704. The recessed well 704 acts as a columnator, only allowing light from a narrow range of angles to be detected, thereby reducing stray signals from other nearby groups having light reflected therefrom. The cDNA 604 or other binding molecules are bound to the ball surface 101 directly on top of, or as near to the photo-detector 702 as possible to ensure that each photo-detector 702 receives a photo signal from just one

light source 701.

ELECTRON TRANSFER SENSOR

Referring now to FIGURE 8, there is illustrated an electron transfer detector. The electron transfer detector 800 is described by Lockhart et al., in U.S. Patent No. 5,770,722 entitled "Surface-Bound, Unimolecular, Double-Stranded DNA," which issued June 23, 1998. With such a detector 800, each oligonucleotide probe 802 is attached to the ball semiconductor 100 on a gate 810 of a multi-gated FET. (Note that a probe is understood to be those molecules that are designed to act like ligands, but where the binding information is yet unknown.) An electron acceptor complex 804 is attached near the distal end of a short spacer chain 806 composed of poly(ethylene oxide), linking the oligonucleotide probe 802 to the semiconductor ball 100. Multiple different oligonucleotide probes 802 each containing an electron acceptor complex 804 are placed on the semiconductor ball 100. It can be appreciated that there can be at least two diverse, unique probes on each ball 100, or substantially larger numbers of between 100 and 10,000 diverse, unique probes on each ball 100. Target DNA strands 801 containing electron donor complex(s) 808 are passed over the ball surface 101 allowing hybridization to occur. Initiation of the electron transfer requires the application of an external light source. In the above sequence, following the surface hybridization reaction, a light is applied to the ball 100. Such a method could be similarly implemented for other binding molecules designed to create such a "binding-exclusive" path needed for electron transfer.

The electron transfer reaction will be detected at a site where hybridization has occurred producing double-stranded, full complementary DNA. Conversely, if there are base-pair mismatches, the observed electron transfer does not occur. Target DNA strands 801 without electron donor complex(s) 808 can also be incubated with semiconductor balls 100 containing oligonucleotide probes 802/electron acceptor complex(s) 804 to induce hybridization. Electron donor complexes 808 are added to the milieu surrounding the semiconductor ball 100 producing an electron transfer reaction at the site(s) of hybridization. The electron transfer will be detected by the FET. A signal will only be generated for those sites where hybridization is complete. Electron transfer occurs simultaneously at all locations where there was complete hybridization, as an electron is transferred from the donor complex 808 to the acceptor

complex 804, and into processing circuits of the semiconductor ball 100.

LUMINESCENCE SENSOR

The third type of sensor (not illustrated) measures the change in inherent luminescence of an electron acceptor bound to single-stranded DNA in an aqueous environment. The electron acceptor becomes brightly luminescent when bound to the single-stranded oligonucleotide in an aqueous environment. If the oligonucleotide hybridizes to a complementary strand, a "pi way" is formed from overlapping pi orbitals, allowing efficient electron transfer. A reduction in the luminescence signal indicates complementary surface hybridization. Such a method could be similarly implemented for other binding molecules designed to create such a "binding-exclusive" path needed for electron transfer.

Referring now to FIGURE 9, there is illustrated a multi-gated FET 902 semiconductor structure of the ball. The ball 100 has a substrate 900 which may be doped p-type or n-type in accordance with particular requirements of the fabrication process. The gates 810 are embedded in an inter-level dielectric layer 904, which dielectric layer 904 extends in either direction to meet respective contacts (906 and 908) over respective n-well regions (910 and 912). The contacts (906 and 908) partially overlay respective oxide regions (914 and 916) provided for isolation. Because these groups are located on the gates 810 of the multi-gated FET 902, electron transfer will be detected and recorded.

Using the disclosed substantially spherically-shaped semiconductors, it is possible to attach either in situ derived molecules such as oligonucleotides to a spacer of oligo(ethylene oxide), or to modify the surface by an aminosilane and attach purified molecules such as cDNA by a process of micro-printing. These spherical semiconductors can then be used as either micro-array processors or molecular probes. The spherical shape of these semiconductors or molecular detection balls facilitates packing in a column format that provides a substantially greater surface area to fluid volume ratio providing increased sensitivity of detection with the arranged probe arrays. The molecular detection balls provide several avenues of detection including fluorescence, electron transfer, and electrochemical sensing. Ball semiconductors as described in the above-referenced, commonly assigned patent applications include the capability to transfer data telemetrically from the ball to a nearby computer. Applying this capability, each ball can individually sense the degree of hybridization for each molecular probe on its surface, and report that information upon radio frequency interrogation. Because

there is no pre-defined limit on the number of balls in a column, this format provides readily expandable capabilities by simply including more molecular detection balls in the column.

Referring now to FIGURE 10, there is illustrated a liquid chromatography column. In the preferred embodiment, the "molecular detection semiconductor balls" 100 are packed into a column 1000 similar to a liquid chromatography column. This packed ball column 1000 provides an improved surface-area-to-void-volume ratio in comparison with a typical flat semiconductor wafer. In a standard format, there is a minimum of a 40-fold improvement in the surface-area-to-liquid-volume ratio compared with the currently available flat semiconductor chips. This offers increased sensitivity and allows for lower detection limits. There is also more total surface area on the beads in a typical liquid chromatography column than on a single flat chip. In a typical embodiment, approximately 100 times more surface area is available on a packed column 1000 of molecular detection balls 100 than on currently available flat chips. This gives the molecular detection balls 100 increased surface area for placement of more sensors allowing for detection of more sequences. Therefore, the geometry and packing conditions of this disclosed architecture offer inherent advantages over currently used devices.

In one embodiment, the sensitivity of the molecular detection ball 100 is enhanced by oscillating a positive and negative charge on the ball surface 101. Analyte molecules such as DNA and RNA which are negatively charged will be attracted to a positively charged surface. This has the effect of binding negatively charged molecules to the surface 101 by electrostatic charge. When the attracted molecule finds a complementary probe attached to the surface 101, hybridization takes place, and that molecule is attached to the probe by chemical attraction. When the ball surface 101 changes to a negative charge, this repels the non-surface hybridized molecule from the surface 101. This surface charge oscillation increases the sensitivity of the gene ball 100 by bringing more analyte molecules into contact with surface probes.

The telemetry capabilities (e.g., using bi-directional radio-frequency transmissions) of these ball semiconductors 100 are described in the above referenced commonly assigned patent applications, and in greater detail hereinbelow. Using these telemetry capabilities, the balls can be interrogated individually, or as groups, and the balls respond with information regarding which sensors detected complimentary base pairing. This information is fed directly into real-time running software for interpretation. This eliminates additional step required by many

currently available "gene chips" to scan the surface.

Referring now to FIGURE 11, there is illustrated a general block diagram of the circuit embodiments of the DNA ball 100 and an external control station 1100. The control system 1100 includes an antenna/coil 1102 that transmits RF power to an antenna/coil 1104 of the ball 100. Power is transported either by RF radiation or by magnetic coupling between the control system antenna/coil 1102 and the ball antenna/coil 1104. The control system 1100 generates RF power with an RF oscillator 1106 coupled to an RF amplifier 1108. The RF amplifier 1108 is coupled to the control system antenna/coil 1102. RF power received at antenna/coil 1104 of ball 100 is rectified and smoothed by an RF rectifier/smoother 1110 coupled to the antenna/coil 1104. The RF rectifier/smoother 1110 converts RF energy to a DC voltage. The DC power is stored in a DC power storage unit 1112, which may be a capacitor, a battery, or the combination thereof. The capacitor of the DC power storage unit 1112 may be included in the smoothing portion of RF rectifier/smoother 1110. A voltage regulator 1114 is coupled to the DC power storage unit 1112 to regulate the DC voltage in order to provide stable voltage for powering the ball 100, for any condition or distance between control system 1100 and the ball 100. The voltage regulator 1114 supplies DC voltage to all circuits of ball 100, in a manner well-known to those skilled in the art.

A control logic circuit 1118 may be configured to control the activity of all the circuits on ball 100. The control logic 1118 may be a microcontroller, a digital signal processor, or any other processor suitable to the size constraints and functions required to be processed. The control logic 1118 interfaces to a memory 1120 for storing information, and reading information therefrom on command from the control system 1100, or perhaps according to an algorithm running in the control logic 1118. One or more sensor regions 1122 measure the hybridization activities on the molecular detection ball 100, and pass the data into an A/D converter 1124 for conversion. The converter 1124 is controlled by the control logic 1118, and connects to an RF modulator 1126 for modulation of the digital data onto an RF carrier signal generated by an RF oscillator 1128 for transmission from the ball 100. The modulated signal from the RF modulator 1126 is amplified using an RF amplifier 1130 to obtain sufficient signal strength for coupling from the ball 100 to the control system 1100.

The frequency of RF oscillator 1128 is preferably not the same as the frequency generated by RF oscillator 1106 of control system 1100. The RF signal produced by RF

oscillator 1128 is modulated with the signal produced by converter 1124 in the RF modulator 1126. The ball 100 may operate under AM, FM, PM, or any other analog and digital modulation methods. The information transmitted from the ball 100 is received at the control system antenna/coil 1102. The received RF signal is detected by an RF detector 1132 and amplified by an RF amplifier 1134. The amplified signal is converted to a digital signal by an A/D converter 1136. The converter 1136 is coupled to control logic 1138 (similar to the control functions provided by the CPU 112 and control logic 1118), which processes the data received from ball 100, and controls a display 1140 and other electrical circuitry of the control system 1100. The display 1140 provides audio and visual signaling to a human operator, with the visual aspect being as simple as an LED, or as complex as a computer display, or it may simply be an interface to other instrumentation equipment.

Referring now to FIGURE 12, there is illustrated a general schematic diagram of the circuit embodiments of the molecular detection ball 100 and the external control station 1100 of FIGURE 11. The ball 100, as described hereinabove, is operable to provide one or more sensor regions 1122 for interfacing with the desired quantitative condition, in this particular discussion, hybridization activities using the sensors discussed hereinabove. The illustrated embodiment is that associated with a "passive" system, which term refers to a system having no battery associated therewith. In order to operate the system, there is provided an inductive coupling element 1204 in the form of an inductor, which is operable to pick up an alternating wave or impulse via inductive coupling, and extract the energy therein for storage in the inductive element 1204. This will create a voltage across the inductive element 1204 between a node 1206 and a node 1208. A diode 1210 is connected between the node 1208 and the node 1212, with the anode of diode 1210 connected to node 1208 and the cathode of diode 1210 connected to a node 1212. Typically, the diode 1210 will be fabricated as a Schottky diode, but can be a simple PN semiconductor diode. For the purposes of this embodiment, the PN diode will be described, although it should be understood that a Schottky diode could easily be fabricated to replace this diode. The reason for utilizing a Schottky diode is that the Schottky diode has a lower voltage drop in the forward conducting direction.

The diode 1210 is operable to rectify the voltage across the inductive element 1204 onto the node 1212, which has a capacitor 1214 disposed between node 1212 and node 1206. Node 1212 is also connected through a diode 1216 having the anode thereof connected to node 1212 and the cathode thereof connected to a node 1218 to charge up a capacitor 1220 disposed

between node 1218 and 1206. The capacitor 1220 is the power supply capacitor for providing power to the ball 100. The capacitor 1214, as will be described hereinbelow, is operable to be discharged during operation of the system and, therefore, a separate capacitor, the capacitor 1220, is required for storing power to power the system of the ball 100.

There is also provided a switching transistor 1231 which has one side of the gate/source path thereof connected to a node 1228 which is the data output of the sensors 1122, and the other side thereof connected to a node 1232. The gate of transistor 1231 is connected to the output of a switch control 1230. Node 1232 is connected to the input of a buffer 1234 to generate an analog signal output thereof which is then converted with an analog-to-digital converter 1236 to a digital value for input to a CPU 1238. The CPU 1238 is operable to receive and process this digital input voltage. A clock circuit 1240 is used for providing timing to the system. The memory 1120 is provided in communication with the CPU 1238 to allow the CPU 1238 to store data therein for later transmittal back to the control system 1100 or for even storing received instructions. This memory 1120 can be volatile or it can be non-volatile, such as a ROM. For the volatile configuration, of course, this will lose all information when power is removed. The CPU 1238 is operable to provide control signals to the switch control 1230 for turning on the transistor 1231 at the appropriate time. In addition to the transistor 1231 being toggled to read the one or more sensors 1122, transistor 1231 could be a pass-through circuit such that the CPU 1238 can continually monitor the voltage at the output of the sensors 1122. System power to all power-consuming elements of the ball 100 is provided at the SYSTEM PWR output (or node 1218).

The memory 1120, in conjunction with the operation of the CPU 1238, can be operated such that a hybridization history can be stored for the one or more sensor regions 1122. Similarly, the hybridization profile history could be stored and later uploaded to the control system 1100 for immediate or subsequent analysis. This would require a time base, which is provided by RF oscillator 1128 (illustrated herein as part of a transmit/receive circuit 1242) and which would comprise an integral part of the operation of the CPU 1238. This allows information in the form of hybridization measurements to be taken at certain times.

In order to communicate with the CPU 1238 for transferring data thereto and for allowing the CPU 1238 to transfer data therefrom, the receive/transmit circuit 1242 is provided for interfacing to node 1212 through a resistive element 1244. This allows RF energy to be

transmitted to node 1212. It is important to note that the semiconductor junction across diode 1210 is a capacitive junction. Therefore, this will allow coupling from node 1212 to node 1208. Although not illustrated, this could actually be a tuned circuit, by selecting the value of the capacitance inherent in the design of the diode 1210. In any event, this allows an RF connection to be provided across diode 1210 while allowing sufficient energy to be input across inductive element 1204 to provide a voltage thereacross for rectification by the diode 1210 and capacitor 1214. Typically, the frequency of this connection will be in the MHz range, depending upon the design. However, many designs could be utilized. Some of these are illustrated in Beigel, U.S. Patent No. 4,333,072, entitled "Identification Device," issued June 1, 1982, and Mogi et al., U.S. Patent No. 3,944,982, entitled "Remote Control System For Electric Apparatus," issued March 16, 1976, which are incorporated herein by reference. With these types of systems, power can continually be provided to the node 1212 and subsequently to capacitor 1220 to allow power to be constantly applied to the ball 100.

The inductive element 1250 is driven by a driving circuit 1252 which provides a differential output that is driven by an oscillator 1106. This will be at a predetermined frequency and power level necessary to couple energy from inductive element 1250 to inductive element 1204. Since this is an external system, the power of the oscillator can be set to a level to account for any losses attributed to distance from the molecular detection ball 100. To allow information to be transmitted, a modulation circuit 1256 is provided which is modulated by a transmitter signal in a block 1258 that allows information to be modulated onto the oscillator signal of the oscillator 1106, which oscillator signal is essentially a "carrier" signal. However, it should be understood that the information that is transmitted to the ball 100 could merely be data information, whereas the CPU 1238 could operate independent of any transmitted information to provide the temperature output. Alternatively, entire control of the ball system 100 could be provided by the transmit signal 1258 and the information carried thereon, since power must be delivered to the illustrated embodiment due to the lack of any independent power in the ball 100.

When the information is received from the ball 100, it is superimposed upon the oscillator signal driving the inductive element 1250. This is extracted therefrom via a detector 1260 which has the output thereof input to a first low pass filter 1262, and then to a second low pass filter 1264. The output of low pass filters 1262 and 1264 are compared using a comparator 1266 to provide the data. The filter 1262 provides an average voltage output,

whereas the filter 1264 provides the actual digital voltage output. The output of the comparator 1266 is then input to a CPU 1270 which also is powered by the oscillator 1106 to process the data received therefrom. This can then be input to the display 1140.

Referring now to FIGURES 13A-C, there are illustrated alternate embodiments for the transmit/receive operation. In FIGURE 13A, there is provided an oscillator 1300 (similar to oscillator 1106) which drives an inductive element 1302. Typically, there is some type of load 1304 disposed across the inductive element 1302. This is the primary power that is provided to the ball system 100. A separate inductive element 1306 is provided on the ball 100, for being inductively coupled to the inductive element 1302. Thereafter, a voltage is generated across the inductive element 1306, the inductive element 1306 being connected between nodes 1308 and 1310. A diode 1312 is connected between node 1308 and a power node 1314, and a power supply capacitor 1316 is disposed across node 1314 and a node 1310. This allows the voltage on node 1308 to be rectified with diode 1312.

Referring now to FIGURE 13B, the receive operation in this alternative embodiment utilizes a separate inductive element or antenna 1324 in the ball 100, which is operable to be connected between nodes 1309 and 1311. Node 1309 is capacitively coupled to a transmit node 1330 with a capacitor 1332, the capacitor 1332 being a coupling capacitor. A transmitter 1334 is provided for transmitting received data from a line 1336 to the node 1330, which is then coupled to the node 1309 to impress the RF signal across the inductive element 1324. A corresponding inductive element 1340 is disposed on the remote controller of control system 1100, which inductive element 1340 is operable to be disposed proximate to the inductive element 1324, or a distance therefrom depending upon the signal power. The inductive element 1340 is basically a "pick-up" element which is operable to receive information and function as an antenna, and provide the received signal to a receiver 1342. The structure of FIGURE 13B is a separate structure, such that node 1309 is isolated from node 1308, the power receiving node. However, it should be understood that any harmonics of the oscillator 1300 would, of course, leak over into the inductive element 1324. This can be tuned out with the use of some type of tuning element 1344 on the ball 100 disposed across inductive element 1324, and also a tuning element 1346 disposed across the inductive element 1340, i.e., the antenna.

Referring now to FIGURE 13C, there is illustrated a simplified schematic diagram of

the receive portion. The ball 100 has associated therewith a separate receive antenna or inductive element 1350 disposed between node 1313 and a node 1352. Node 1352 is capacitively coupled to a receive node 1354 with a coupling capacitor 1356. A receiver 1358 is provided for receiving the information transmitted thereto and providing on the output thereof data on a data line 1360. The receiver 1358 is operable to receive the RF signal, demodulate the data therefrom, and provide digital data on the output 1360. A transmitter 1362 is operable to impress a signal across an external inductive element 1364. The inductive element 1364 basically provides the RF energy and is essentially tuned with a tuning element 1366. A corresponding tuning element 1368 is provided on the ball 100 and disposed across inductive element 1350, the inductive element 1350 acting as an antenna, as well as the inductive element 1364.

Note that in circumstances where the signals of ball 100 cannot be adequately received therefrom and/or power coupled thereto, the signal coupling head of the control system 1100 may need to be placed proximate to the ball 100 in order to couple the transmit/receive signals and power. Furthermore, where more than one ball 100 is used, as in aggregate clusters (discussed in greater detail hereinbelow), communication of power and data signals between the various balls 100 may need to employ distinct time periods (i.e., time multiplexing) when communication occurs using a single common frequency, or discrimination circuits may need to be used where communication occurs simultaneously with the plurality of implanted balls 100 having different oscillator frequencies.

Referring now to FIGURE 14, there is illustrated a physical diagram of a molecular detection ball 100 and associated exposed circuit blocks. The ball 100 comprises a substrate 1400 (similar to substrate 900) upon which the numerous onboard circuit elements are fabricated. Communication and power coils L_1, L_2, and L_3 are provided and oriented substantially orthogonally to one another for coupling energy and signals to the circuits of the ball 100 (when in any orientation) and transmitting signals therefrom. Notably, fewer or more than three coils can be implemented, in accordance with the particular application. In one instance, the coils L_1, L_2, and L_3 are connected to a power regulator 1402, and respective control switches 1404, 1406, and 1408, which control switches 1404, 1406, and 1408 are controlled by the microprocessor 1438. As will be discussed hereinbelow, the three coils L_1, L_2, and L_3 also connect to the transmit/receive circuit 1440 for facilitating communication with external systems which receive and process the sensor data. Though not illustrated, the power

regulator 1402 can also connect to the one or more sensor regions (1416, 1418, and 1420) to provide regulated power for the active circuit components (e.g., photo emitter/detectors of the fluorescence sensor 700, and the multi-gated FETs of the electron transfer sensor 800), or the regulated power can be provided from the regulator 1402 through the microprocessor 1438 to the desired sensor regions 1416, 1418, and 1420.

The microprocessor 1438 provides monitor and control functions for all activities on the molecular detection ball 100. The control switches 1404, 1406, and 1408 can be controlled by the microprocessor 1438 to provide power directly from the respective coils L_1, L_2, and L_3 to active elements of the respective sensor regions 1416, 1418, and 1420. When operable in this manner, the microprocessor 1438 connects to and controls the three switches 1404, 1406, and 1408 to control the amount of energy coupled from each of the respective coils L_1, L_2 and L_3 to respective sensor regions 1416, 1418, and 1420. It can be appreciated that the microprocessor 1438 can be programmed either internally, or externally from a control system (not shown) to cycle power to each of the sensor regions 1416, 1418, and 1420 in a predetermined fashion. For example, energy switched in the form of current to sensor region 1416 may be cycled once every time period, while current switched to sensor region 1418 is switched ten times per the same time period, and current switched to sensor region 1420 is switched twenty-five times per the same time period. This flexibility offers more accurate and effective control of energy being applied by the ball 100.

The power regulation circuit 1402 connects to each of the unswitched sides of the coils L_1, L_2 and L_3 to obtain the maximum power transmitted. For example, if the orientation of the ball 100 is such that the coupled power signal is the greatest on coil L_3, yet weaker on coils L_1 and L_2, the maximum power is still obtainable. Had the power regulator 1402 been connected to only a single coil, the amount of power coupled to the ball 100 would be problematic based upon the orientation of the coils in the electric field provided by the external control system. As mentioned hereinabove, the power regulator 1402 provides power to all onboard circuits during operation of the ball 100.

Each sensor region 1416, 1418, and 1420 connects to the microprocessor 1438 for power (one of several ways mentioned hereinabove for receiving power), A/D conversion, and processing of the measured data. The microprocessor 1438 is illustrated as comprising the A/D function of the A/D 1236, which combined functions can be found in conventional digital

signal processing (DSP) circuits. With power applied to the one or more sensor regions 1416, 1418, and 1420, the microprocessor 1438 can then read the values of the respective sensor regions 1416, 1418, and 1420 to obtain the data produced as a result of the hybridization processes. This data is then converted to the digital regime and transmitted to the external control system for processing.

The RF transmit/receive circuit 1440 connects to the microprocessor 1438 to provide I/O functions for RF signals coming into the ball 100 from the control system 1100, and for the transmission of communication signals from the ball 100 to the control system 1100. The RF circuit 1440 is illustrated as having a single connection to coil/antenna L_2, when in practice it could be connected to any or all three coils L_1, L_2 and L_3 to ensure adequate reception and signal transmission strength to the control system 1100. The RF transmit/receive circuit 1440 can also obtain power through the connection from the microprocessor 1438, or have its own dedicated connection (not shown) from the power regulator circuit 1402. Note that the coils L_1, L_2 and L_3 are used for power coupling and signal communication between the ball 100 and the control system. Therefore, the communication signal may be modulated into the power signal to provide a more continuous exchange of power and data signals. Additionally, the number of coil windings of the coils L_1, L_2 and L_3 can be varied according to the required power levels.

A memory 1442 (similar to memory 1120) connects to the microprocessor 1438, is non-volatile, and stores, for example, a unique ID of the ball 100. The unique ID can be accessed upon command from the control system 1100 for tagging the received data with the particular ball 100 from which the data was obtained, or to send selected signals to selected balls 100 according to the respective unique IDs. It can be appreciated that the memory 1442 can be programmed according to the user's needs. For example, in addition to the unique ID, the memory 1442 may contain information related to the patient, such as name, address, date of usage of the ball 100, type of DNA test performed, the attending physician and hospital, circumstances under which the ball was used (e.g., DNA testing), etc.

In another aspect of the disclosed architecture, and which will be discussed in greater detail hereinbelow, where an aggregate of balls 100 are used, a subgroup of the ball aggregate may be programmed with a common ID such that during operation, that subgroup aggregate of balls 100 may be energized, while others are not. This feature may be used where more than

one aggregate is implemented in testing, each aggregate used for specific purposes, and under perhaps different test conditions. Notably, the unique ID can be programmed at the site by the control system prior to introduction of the ball 100 and/or aggregate into the particular application.

Referring now to FIGURE 14A, there is illustrated a cluster of molecular detection balls in an addressable array. The balls are connected to each other by gold bump interconnects or other suitable interconnective techniques; the interconnects are insulated from the bathing fluid, for example by a polyxylylene coating or other suitable coatings. Information about the binding state of each detection site on each ball is transmitted to an external signal processor by direct electronic coupling, or by an RF transmitter/detector. A continuous flow of fluid through the interstices of the column array of ball detectors brings the analyte in close approximation to the array of hybridization sites. As hybridization occurs, signals are transmitted to the external processor where analysis and/or process control steps are effected by conventional techniques.

Referring now to FIGURE 15, there is illustrated a cross section of a molecular detection ball. The ball 100 preferably comprises the spherical-shaped semiconductor substrate 1400 on which an integrated circuit has been formed, and which may be doped with p-type or n-type impurities in accordance with the particular requirements of the fabrication process. Semiconductor circuitry, indicated generally at 1545, resides on substrate 1400, and includes the power regulator 1440, the RF interface circuitry 1402 with mixing circuit and amplifier, as well as other circuitry. The substrate 1400 and circuitry 1545 are covered by an insulating layer 1547. Insulating layer 1547 is preferably formed of silicon dioxide or phosphosilicate glass. A sensor region 1525 (similar to sensor regions 1416, 1418, and 1420) is disposed near the surface of the ball to facilitate attachment of, for example, the spacer chains 806 (in the application of the electron transfer sensor 800) and the fabrication of the photo emitter/detector pairs (for the fluorescence sensor 700). Suitable connections are provided through the insulating layer 1547 to circuitry 1545.

A power and transmit/receive coil 1521 (only one shown, and similar to each coils L_1, L_2 and L_3, and antenna/coil 1204) is formed of helically-wrapped windings over the insulating layer 1547. The power coil 1521 may have any number of individual windings 1522 which can be fabricated from a deposited layer of aluminum that is patterned and etched using

conventional semiconductor fabrication techniques. The actual number of individual windings of power coil 1521 may be far greater than the six illustrated. The ball 100 is coated in selected regions with or encapsulated in a layer 1549 of biologically inert material such as phosphosilicate glass in order to withstand the various cleaning or chemical processes required implement the disclosed architecture.

Referring now to FIGURE 16, there is illustrated a side view of an alternative embodiment utilizing additional circuitry or structure attached to the ball 100 for providing a local power source. As described hereinabove, the ball 100 requires a power-generating structure for storing a power supply voltage such that diodes must be provided for receiving and rectifying a large amount of power and charging up a power supply capacitor. Alternatively, the ball 100 could be configured to interface to an attached power supply system 1600 comprising either a battery or a capacitor, or both. The local power supply system 1600 is illustrated as disposed on a circuit board 1603 defined by supporting structures 1602 and 1604. The circuit board 1603 contains electronics for interfacing the local power supply system 1600 to the ball 100. The entire structure of FIGURE 16 would be encapsulated, with only a thin layer thereof disposed over ball 100.

Referring now to FIGURE 17, there is illustrated a side elevation of a cluster 1700 of semiconductor gene balls 100 that may be employed in a sensor function, according to a disclosed embodiment. Although a single ball 100 can include the foregoing functions, more complex monitoring functions with multiple sensors can be implemented using multiple ball systems according to the disclosed architecture, or attached to catheters, needles and other insertable devices. For example, the cluster 1700 can include a ball 1781 for power receiving and data transmission functions. Alternatively, ball 1781 can be a miniature battery. A ball 1782 can include a first sensor function, such as pressure sensing, and a ball 1783 can include a second sensor function, such as measuring pH, pO_2, pCO_2, or temperature, as the particular application requires. Connections between the balls are made through metal contacts 1790, which may be solder bumps.

Referring now to FIGURE 17A, there is illustrated a conventional block diagram of the molecular detector coupled to an actuator for continuous process control of an analyte flow. A portion of the analyte flow is sampled by a molecular detector. Based upon the particular makeup of the analyte flow, the molecular detector then provides input to a comparator via

either radio frequency transmission or direct cable connection. The comparator compares the received detector information against known parameters, and outputs a value to a flow control circuit which processes the received information to generate a control signal in accordance with the comparator output information. The control signal is then used to control the flow control mechanism during continuous process control, which then directs output of the flow control mechanism to any of a number of separate bins (Bin A, Bin B, or Bin C) based upon the makeup of the analyte flow.

Referring now to FIGURE 18, there is illustrated a cross section taken along the line 18-18 of FIGURE 17 to expose the four contacts 1888a, 1888b, 1888c and 1888d between ball 1782 and ball 1783. The contacts 1888a and 1888b may be power contacts, such as a positive 3.0 volts and ground, which can be passed from ball 1781 around ball 1782 by conductors on its surface using two of a group of similar contacts (designated collectively by numeral 1790 in FIGURE 17). The contacts 1888c and 1888d may be data and control contacts for communications between ball 1782 and ball 1783. Similarly, data and control contacts may exist among contact group 1790 between ball 1781 and ball 1782 to the extent needed.

Referring now to FIGURE 19, there is illustrated a cluster or aggregation 1900 of balls 1991, 1992, 1993, 1994, 1995 and 1996, as an example of the versatility of such ball systems. The cluster 1900 specifically shows six balls arranged in a three-dimensional configuration. It will be appreciated that various other cluster arrangements are possible, limited only by the constraints of the end-use application. Each of the balls (similar to ball 100) of the cluster 1900 can perform different functions and communicate with each other through contacts as described above in connection with FIGURES 17 and 18. For example, ball 1996 may serve as a battery ball to provide power for the remaining balls of the cluster 1900, ball 1995 may be a sensor ball used specifically for electron transfer sensing functions, and ball 1994 may be implemented strictly for fluorescent sensing, etc. Clustered balls are able to integrate, transmit, and receive more complex information or actuate a response (emit laser, infrared, ultrasound, or electrical energy). The actuators may contain a piezoelectric driver attached to a ball surface for ultrasound generation and control for measurements of luminal diameter and fluid flow rate within a vessel lumen. Such actuators can serve as an emitting device allowing for external detection to determine location or position.

Referring now to FIGURE 20, there is illustrated more detailed semiconductor

structures the emitter/detector pairs of the fluorescence sensor. The LED structure 701 emits light 2000 (or other energy) out an emitter well 2002 which impinges on a molecular structure 2001. The molecular structure 2001 releases photo energy 2004 which is detected by the photo-detector structure 702 buried in a detector well 2006 proximate to the emitter well 2002. The LED structure 701 is commonly known, and a wide variety of structures may be employed to obtain the desired results. For example, underlying the glass passivation layer 2008 (used for isolation of the ball electronics from the chemicals used in accordance with the disclosed architecture) are metal contacts 2010 which contact and partially overlay a diffused region 2012. The diffused region 2012 may be a p^+ region diffused in an n-type region 2014 which overlies the more heavily n^+-doped substrate 1400. Note that the photo structures are not limited to diodes, but may also be phototransistor structures.

The detector structure 702 is also commonly known, and can be formed using conventional deposition and fabrication technique practices. For example, underlying the passivation layer 2008 are metal contacts 2016 for electrical interfacing. Underlying the metal contacts 2016 is an oxidation layer 2018 (e.g., SiO_2). The metal contacts 2016 partially overlay and contact a diffused region 2020, which may be a p^+ region, in this particular embodiment. Underlying the p^+-doped region 2020 lies an n-doped region 2022, followed by the substrate 1400, which may be a more heavily doped n^+ region.

Referring now to FIGURE 21, there is illustrated a conventional circuit block diagram of the photo emitter/detector circuits as fabricated and illustrated in FIGURE 20. An emitter circuit 2102 comprises the emitter structure 701 and LED interface electronics 2140 which couples to the emitter structure 701 for power and control thereof. In operation, the emitter interface electronics 2140 drives the LED 2120 to emit light 2000 which impinges on the molecular structures and is ultimately detected by the detector structure 702. The photocoupler 702 outputs a voltage in proportion to the light intensity received from the impinged molecular structure, which voltage signals are fed into respective coupler interface electronics 2142. The change in intensity of the detected light then provides a measure of the hybridization which has occurred on the impinged molecular structure.

In summary, techniques for rapid molecular analysis are disclosed that employ oligonucleotides or other specific molecular arrays covalently attached to the surface of one or more miniature substantially spherically-shaped semiconductor devices. When these devices

are exposed to a fluid medium containing biological samples with target molecules, the target molecules will spontaneously hybridize onto the surface at those locations where complementary binding can take place between the surface probes and the sample molecules. These devices can then identify the exact location where surface hybridization has taken place using one or more distinct detectors incorporated into the spherical semiconductor, including: electrochemical, electron transfer, or fluorescent detectors. By correlating the precise position of a detected signal to the known molecular probe at that position, the molecules that hybridize to the surface can be immediately and uniquely identified. Methods for attaching the molecular probes and the required circuitry for reporting the results of the molecule detection to a nearby computer by radio-frequency transmission or direct electrical connection are herein disclosed. Furthermore, the detected signals from single balls or ball arrays can be transmitted to actuators for continuous or batch process control steps, including fluid switching, pumping, filtration, heat and/or mass transfer, etc. These steps enable for controlled processing of DNA, RNA and other molecules in industrial, agricultural and clinical settings.

Although the preferred embodiment has been described in detail, it should be understood that various changes, substitutions and alterations can be made therein without departing from the spirit and scope of the invention as defined by the appended claims.

WHAT IS CLAIMED IS:

1. A method of molecular analysis, comprising the steps of:

providing one or more molecule-receptive surface regions on the surface of a spherical-shaped semiconductor device;

exposing the semiconductor device to a medium containing molecules from a controlled source;

causing the molecules to bond to any of the one or more molecule-receptive surface regions of the semiconductor device;

sensing bonding information when the molecules bond at each of the one or more molecule-receptive regions; and

transmitting the bonding information to a computer for processing.

2. The method of Claim 1, wherein the spherical-shaped semiconductor device in the step of providing has a diameter of approximately one millimeter or less.

3. The method of Claim 1, wherein the molecule in the step of causing are bonded to the surface by a hetero-bifunctional oligomer of polyethylene oxide.

4. The method of Claim 3, wherein the oligomer of polyethylene oxide contains one end group for coupling to the surface of the spherical-shaped semiconductor device, and one end group that only contains a photo-activatable moiety.

5. The method of Claim 3, wherein the hetero-bifunctional oligomer of polyethylene oxide distally terminates in both an electron acceptor and a photo-activatable end group.

6. The method of Claim 5, wherein the electron acceptor is Rh phi sub 2 phen sup 3 plus phi, phenanthrenequinone diimine.

7. The method of Claim 1, wherein there are at least two diverse, unique probes on each spherical-shaped semiconductor device.

8. The method of Claim 1, wherein there are between 100 and 10,000 diverse,

unique probes on each spherical-shaped semiconductor device.

9. The method of Claim 1, wherein the providing step further includes providing a plurality of the spherical-shaped semiconductor devices, each having molecule-receptive surface regions thereon.

10. The method of Claim 1, wherein the instance of oligonucleotides the molecules in the step of causing are attached from the 3′ to the 5′ direction.

11. The method of Claim 1, wherein the instance of oligonucleotides the molecules in the step of causing are attached from the 5′ to the 3′ direction.

12. The method of Claim 1, wherein the molecules in the step of causing are attached directly to a gate of a field effect transistor of the spherical-shaped semiconductor device.

13. The method of Claim 1, wherein the molecules in the step of causing are attached on or adjacent to an emitter/detector combination of the spherical-shaped semiconductor device.

14. The method of Claim 1, wherein the molecules are designed to bind RNA.

15. The method of Claim 1, wherein the molecules bind to single-stranded genomic DNA, PCR product, or like molecules.

16. The method of Claim 1, further comprising the step of applying an aminoalkylsilane coating to the surface of the spherical-shaped semiconductor device.

17. A method of rapid gene analysis, comprising the step of linking cDNA fragments directly to the surface of one or more spherical-shaped semiconductor devices.

18. The method of Claim 17, wherein the one or more spherical-shaped semiconductor devices are coated with an aminoalkylsilane.

19. The method of Claim 17, wherein the one or more spherical-shaped semiconductor devices are approximately one millimeter or less in diameter.

20. The method of Claim 17, wherein the cDNA fragments are tethered to the surface with linking molecules rather than being directly linked to the surface.

21. The method of Claim 20, wherein the linking molecules are oligomers of polyethylene oxide molecules.

22. The method of Claim 21, wherein the polyethylene oxide molecules are terminated with one or more photo-activating groups.

23. The method of Claim 17, wherein the one or more spherical-shaped semiconductor devices are capable of imposing a net positive surface charge to attract target molecules.

24. The method of Claim 17, wherein one or more spherical-shaped semiconductor devices in an addressable packed column array are capable of detecting single or multiple analytes in continuous fashion.

25. The method of Claim 17, wherein signals obtained from single or multiple analytes detected by one or more spherical-shaped semiconductor devices in an addressable packed column array are capable of controlling processes in a fluid stream in batch mode fashion.

26. The method of Claim 17, wherein signals obtained from single or multiple analytes detected by one or more spherical-shaped semiconductor devices in an addressable packed column array are capable of controlling processes in a fluid stream in continuous flow fashion.

27. A system of rapid gene analysis, comprising:

one or more oligonucleotide-receptive surface regions on the surface of a spherical-shaped semiconductor device; and

a medium containing oligonucleotides from a controlled source to which said semiconductor device is exposed;

wherein said oligonucleotides are caused to bond to any of said one or more oligonucleotide-receptive surface regions of said semiconductor device;

wherein bonding information is sensed when said oligonucleotides bond at each of said one or more oligonucleotide-receptive regions, and said bonding information is transmitted to a computer for processing.

28. The system of Claim 27, wherein said spherical-shaped semiconductor device has a diameter of approximately one millimeter or less.

29. The system of Claim 27, wherein said oligonucleotides are bonded to the surface by a hetero-bifunctional oligomer of polyethylene oxide.

30. The system of Claim 27, wherein information is sensed when said oligonucleotides bond at one or more receptor regions in a calibrated, addressable array of molecular detection semiconductor balls.

31. The system of Claim 27, wherein information sensed at one or more receptor regions in a calibrated, addressable array of molecular detection semiconductor balls is used to control process variables in an industrial production fluid stream.

32. The system of Claim 27, wherein information sensed at one or more receptor regions in a calibrated, addressable array of molecular detection semiconductor balls is used to control process variables in a clinical sample-containing fluid stream.

33. The system of Claim 27, wherein information sensed at one or more receptor regions in a calibrated, addressable array of molecular detection semiconductor balls is used to control process variables in an agricultural process fluid stream.

34. The system of Claim 29, wherein said oligomer of polyethylene oxide contains one end group for coupling to the surface of said spherical-shaped semiconductor device, and one end group that only contains a photo-activatable moiety.

35. The system of Claim 29, wherein said hetero-bifunctional oligomer of polyethylene oxide distally terminates in both an electron acceptor and a photo-activatable end group.

36. The system of Claim 35, wherein said electron acceptor is Rh phi sub 2 phen sup 3 plus phi, phenanthrenequinone diimine.

37. The system of Claim 27, wherein there are at least two diverse, unique probes on each said spherical-shaped semiconductor device.

38. The system of Claim 27, wherein there are between 100 and 10,000 diverse, unique probes on each said spherical-shaped semiconductor device.

39. The system of Claim 27, wherein each of a plurality of said spherical-shaped semiconductor devices are provided having said oligonucleotide-receptive surface regions thereon.

40. The system of Claim 27, wherein said oligonucleotides are attached from the 3′ to the 5′ direction.

41. The system of Claim 27, wherein said oligonucleotides are attached from the 5′ to the 3′ direction.

42. The system of Claim 27, wherein said oligonucleotides are attached directly to a gate of a field effect transistor of said spherical-shaped semiconductor device.

43. The system of Claim 27, wherein said oligonucleotides are attached on or adjacent to an emitter/detector combination of said spherical-shaped semiconductor device.

44. The system of Claim 27, wherein said oligonucleotides are designed to bind RNA to their surface.

45. The system of Claim 27, wherein said oligonucleotides bind to single-stranded genomic DNA, PCR product, or other segments of DNA.

46. The system of Claim 27, wherein an aminoalkylsilane coating is applied to the surface of said spherical-shaped semiconductor device.

47. A system of rapid gene analysis, comprising one or more cDNA fragments linked directly to the surface of one or more spherical-shaped semiconductor devices.

48. The system of Claim 47, wherein said one or more spherical-shaped semiconductor devices are coated with an aminoalkylsilane.

49. The system of Claim 47, wherein said one or more spherical-shaped semiconductor devices are approximately one millimeter or less in diameter.

50. The system of Claim 47, wherein the cDNA fragments are tethered to the surface with linking molecules rather than being directly linked to the surface.

51. The system of Claim 50, wherein said linking molecules are oligomers of polyethylene oxide molecules.

52. The system of Claim 51, wherein said polyethylene oxide molecules are terminated with one or more photo-activating groups.

53. The system of Claim 47, wherein said one or more spherical-shaped semiconductor devices are capable of imposing a net positive surface charge to attract DNA or RNA molecules.

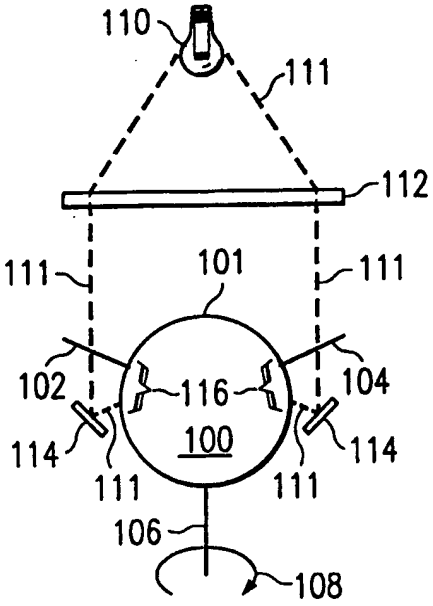


FIG. 1

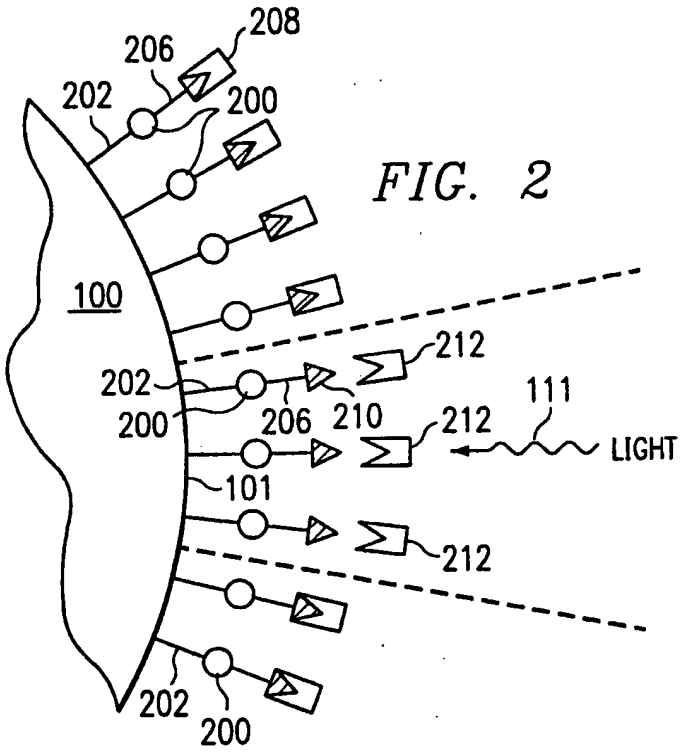


FIG. 2

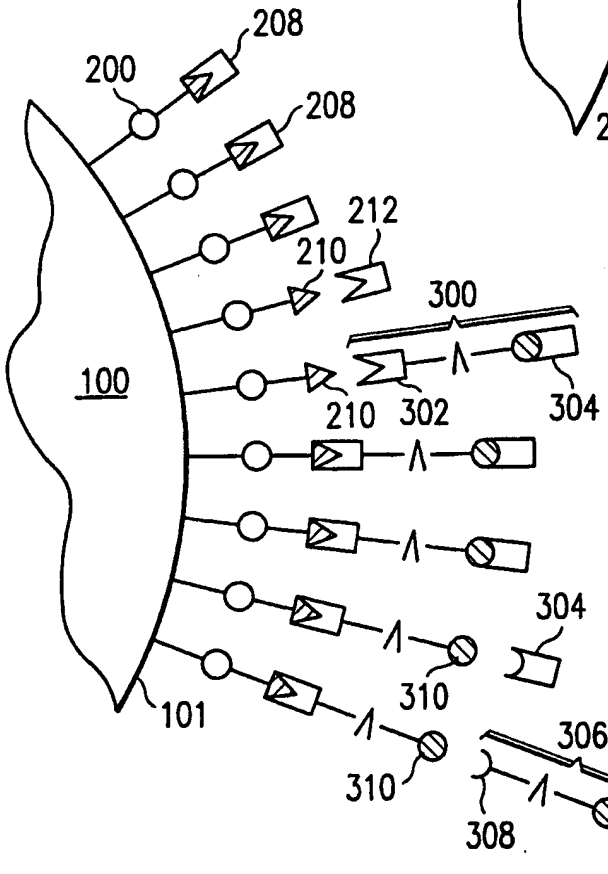
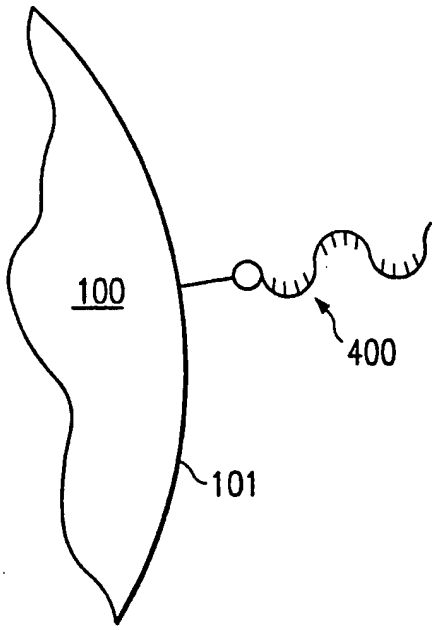


FIG. 3

FIG. 4



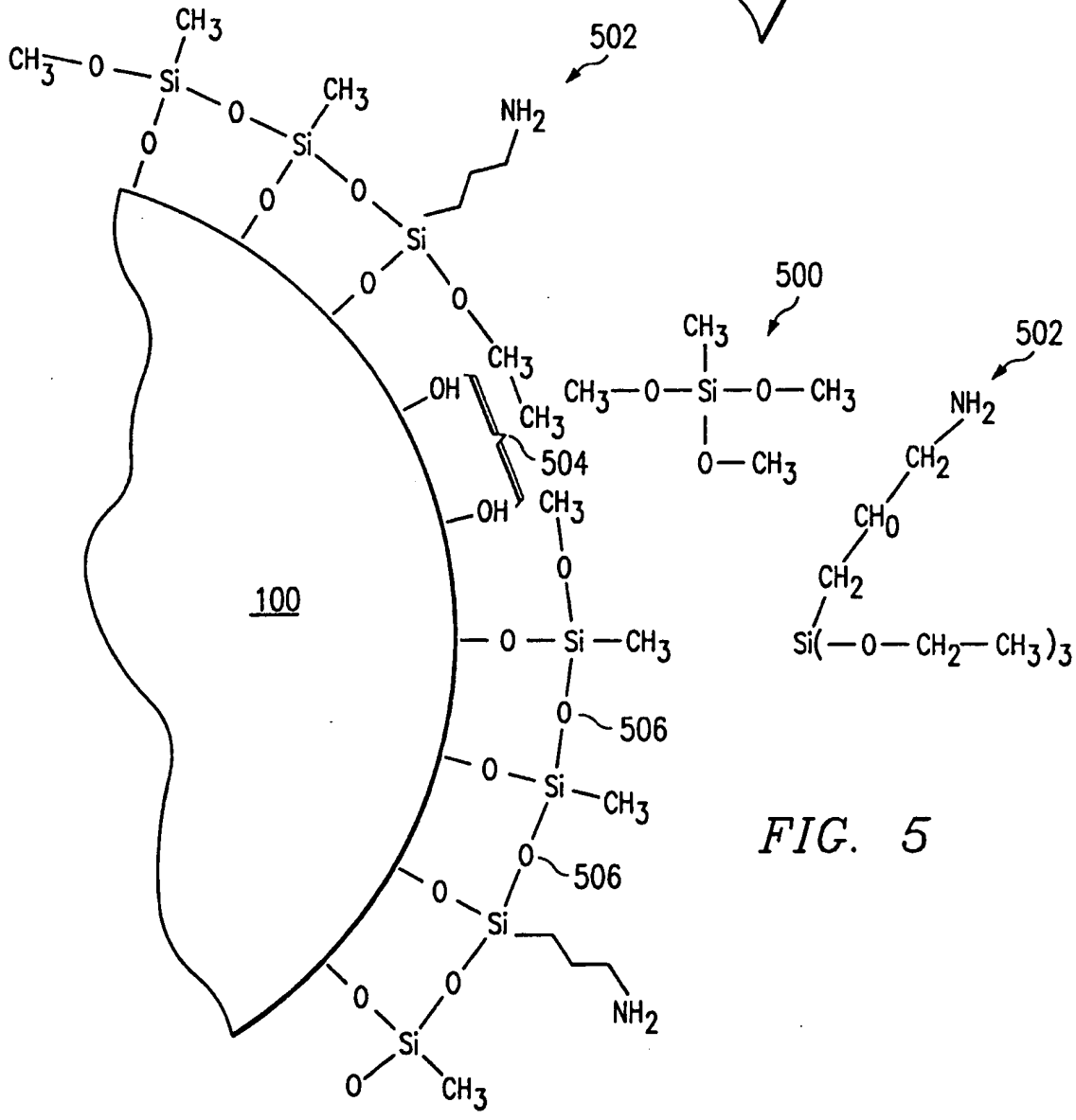


FIG. 5

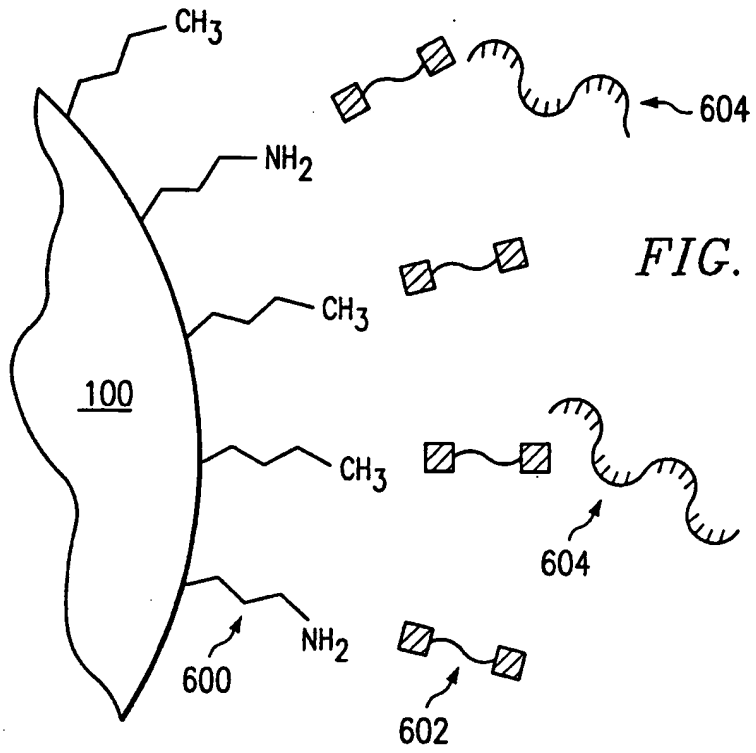


FIG. 6

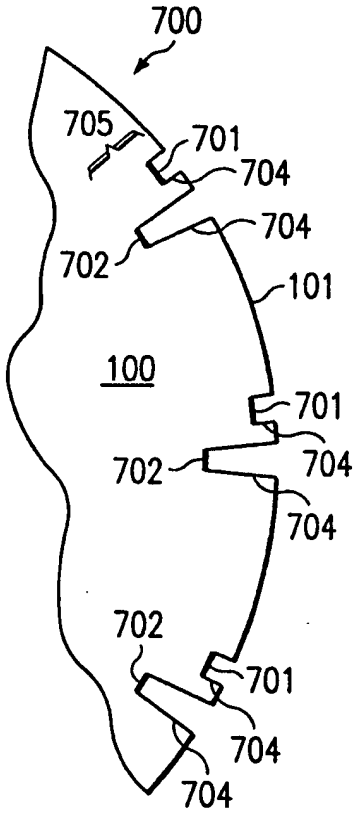


FIG. 7

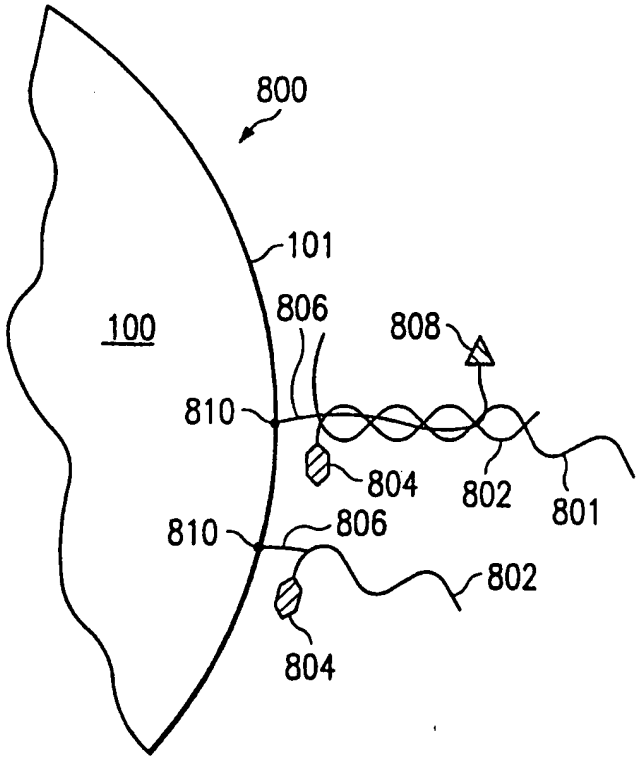


FIG. 8

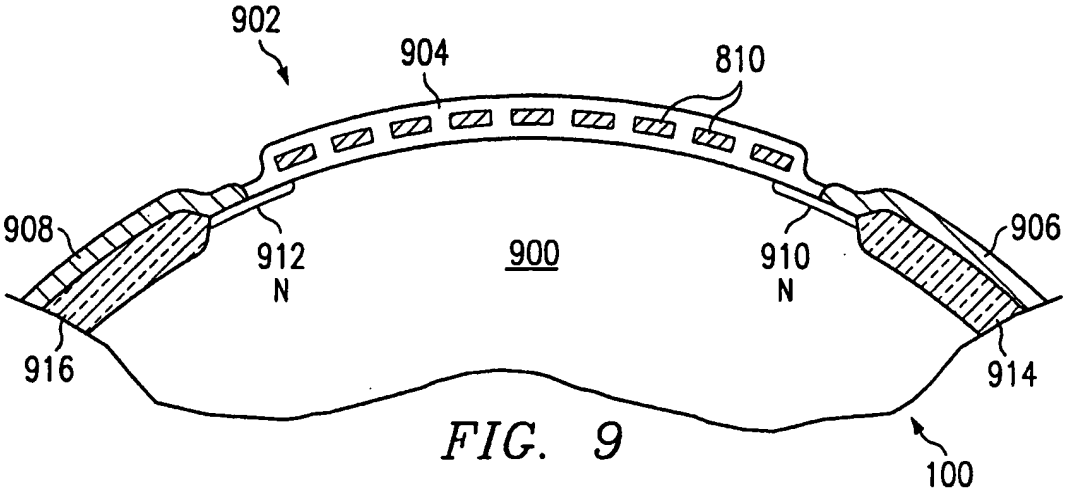


FIG. 9

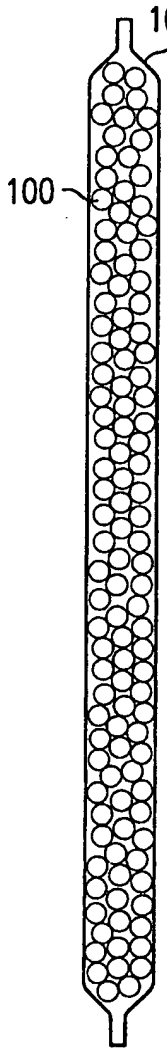
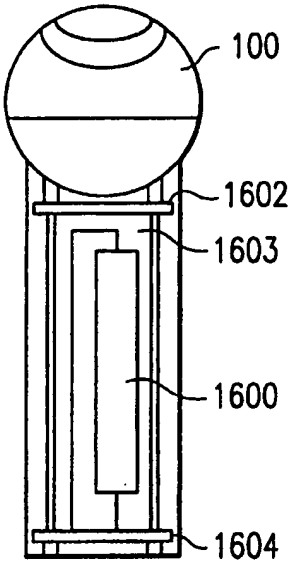


FIG. 10

FIG. 16



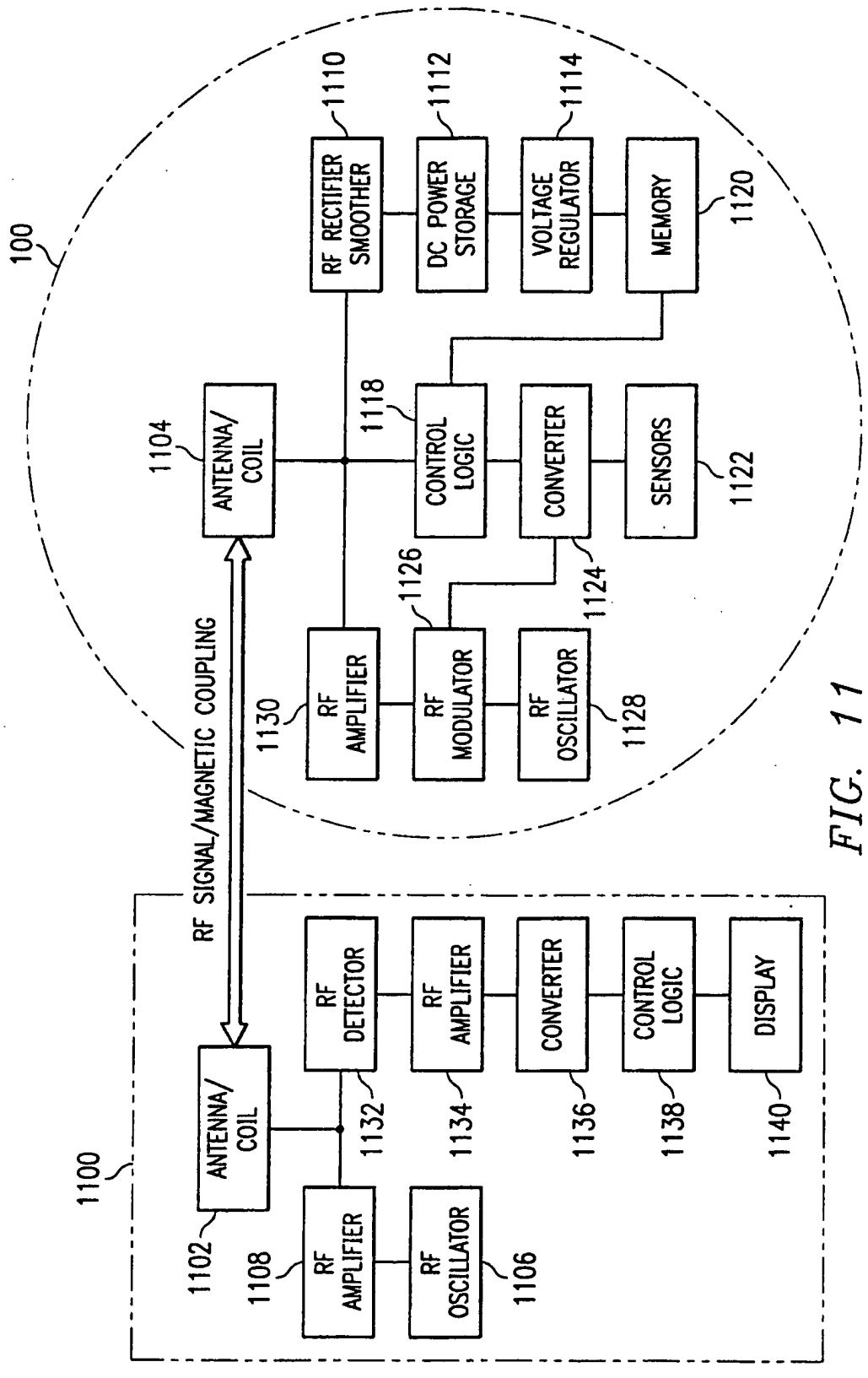

100
1110
RF RECTIFIER SMOOTHER
1112
DC POWER STORAGE
1114
VOLTAGE REGULATOR
MEMORY
1120
1104
ANTENNA/ COIL
1118
CONTROL LOGIC
CONVERTER
SENSORS
1122
1126
1124
1130
RF AMPLIFIER
RF MODULATOR
RF OSCILLATOR
1128
RF SIGNAL/MAGNETIC COUPLING
1100
1102
ANTENNA/ COIL
RF DETECTOR
RF AMPLIFIER
CONVERTER
CONTROL LOGIC
DISPLAY
1132
1134
1136
1138
1140
1108
RF AMPLIFIER
RF OSCILLATOR
1106

FIG. 11

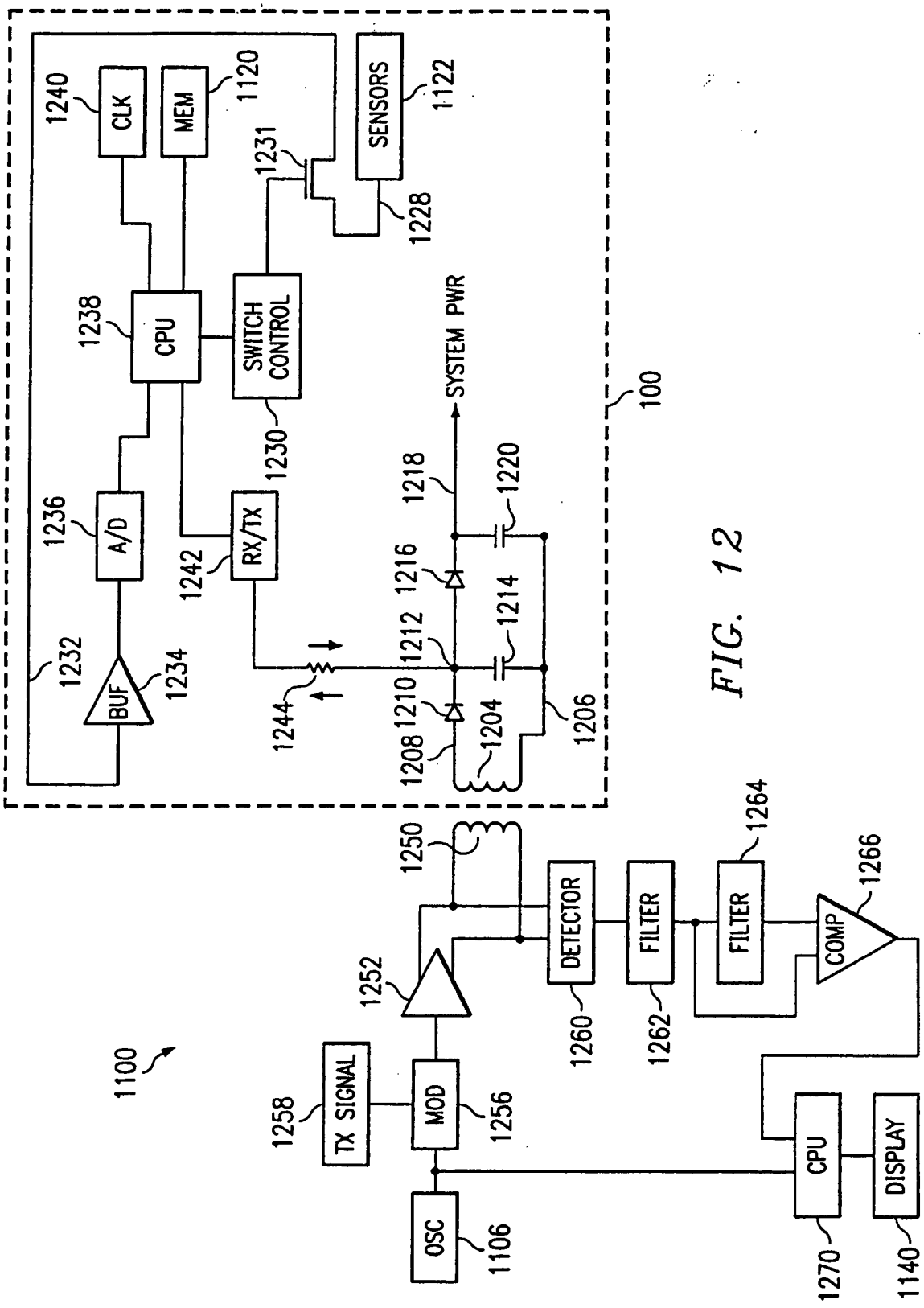

1100
100
1240
CLK
MEM
1120
SENSORS
1122
1231
1228
1238
CPU
SWITCH CONTROL
SYSTEM PWR
1236
A/D
1242
RX/TX
1230
1218
1220
1216
1214
1212
1232
BUF
1234
1244
1210
1208
1204
1206
1250
1252
DETECTOR
FILTER
FILTER
1264
COMP
1266
1260
1262
1258
TX SIGNAL
MOD
1256
OSC
1106
CPU
DISPLAY
1270
1140

FIG. 12

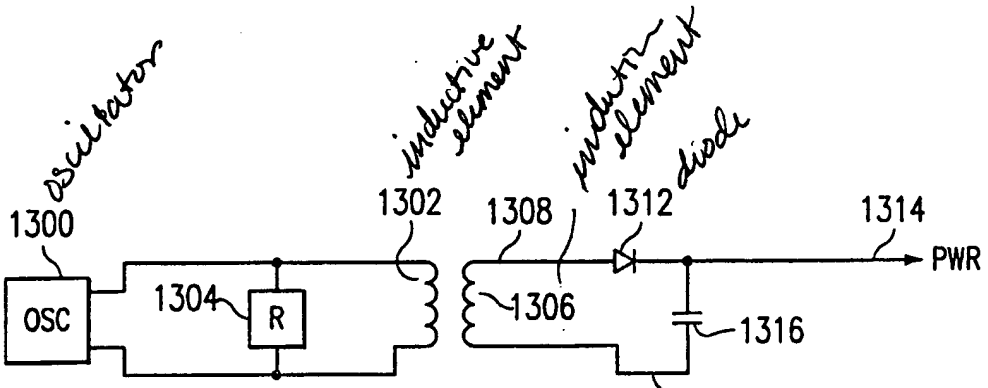


FIG. 13A

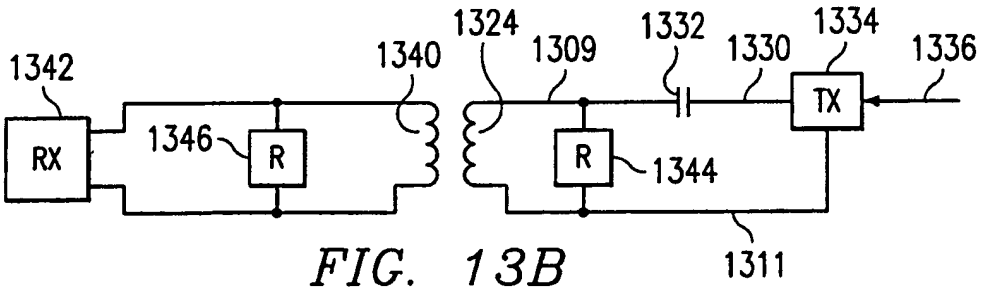


FIG. 13B

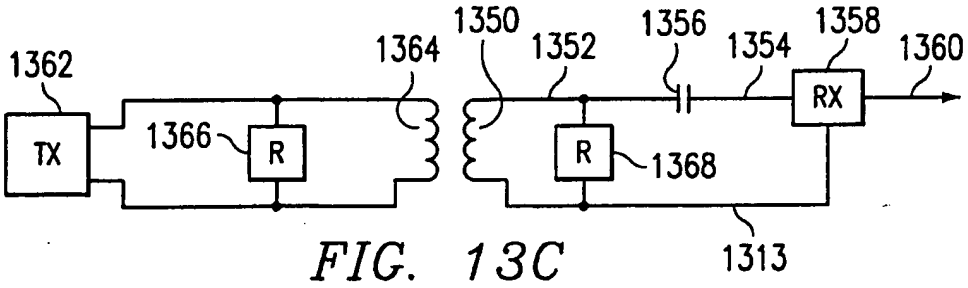


FIG. 13C

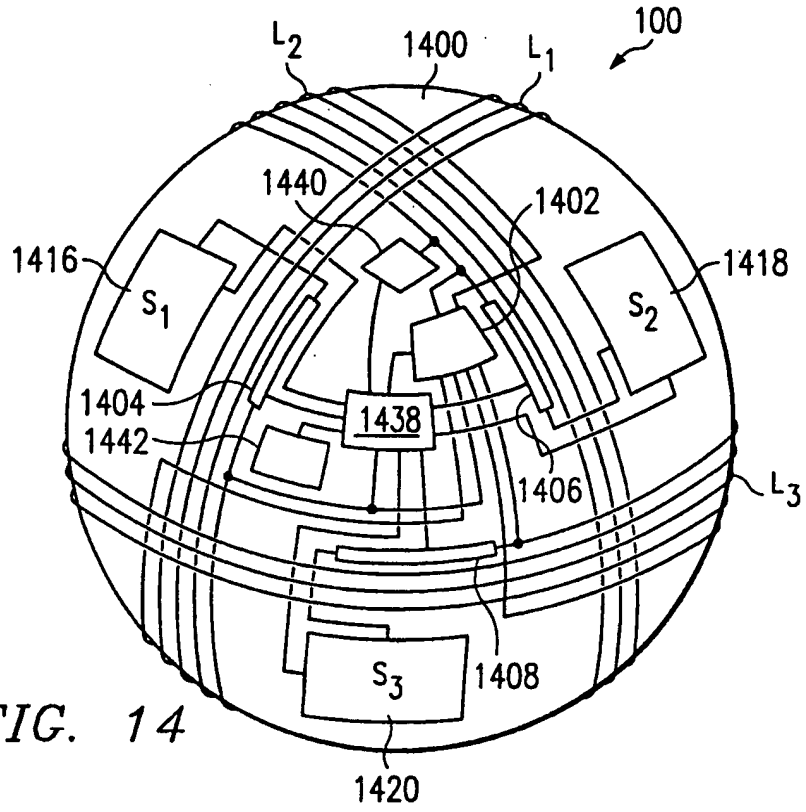

100
L_2
1400
L_1
1440
1402
1416
1418
S_1
S_2
1404
1438
1442
1406
L_3
S_3
1408
1420

FIG. 14

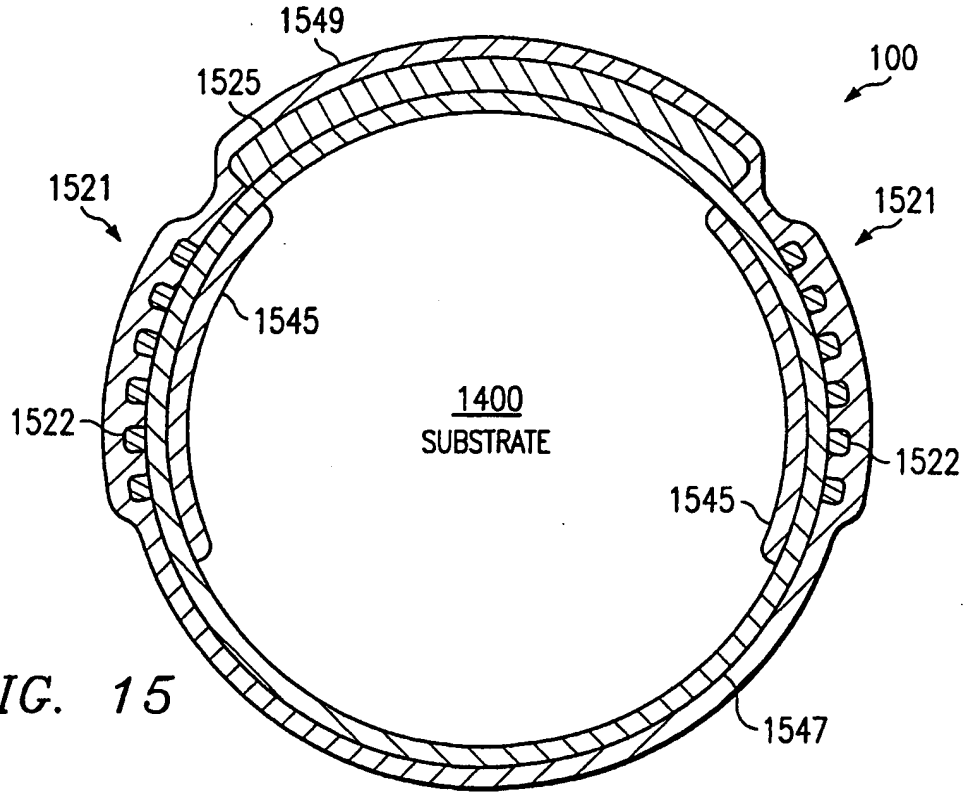

1549
100
1525
1521
1521
1545
1400
SUBSTRATE
1522
1522
1545
1547

FIG. 15

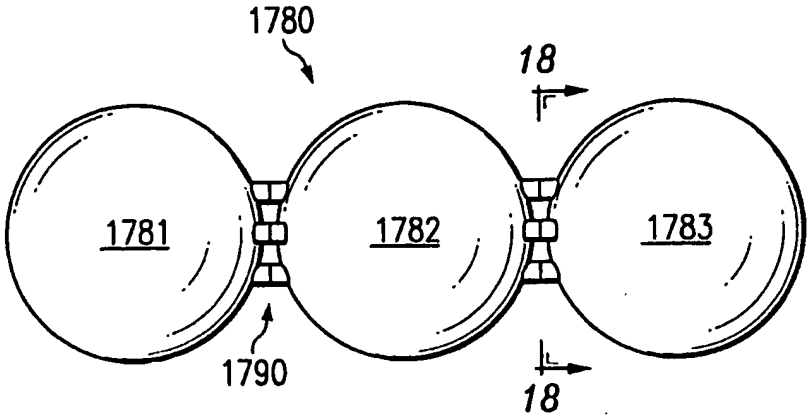


FIG. 17

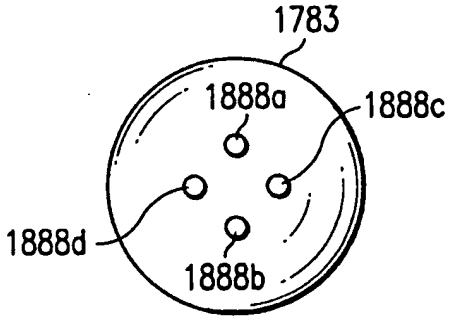


FIG. 18

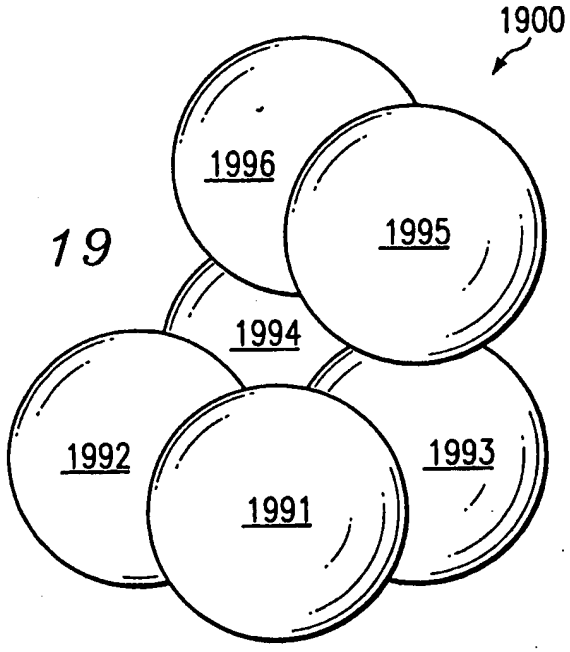


FIG. 19

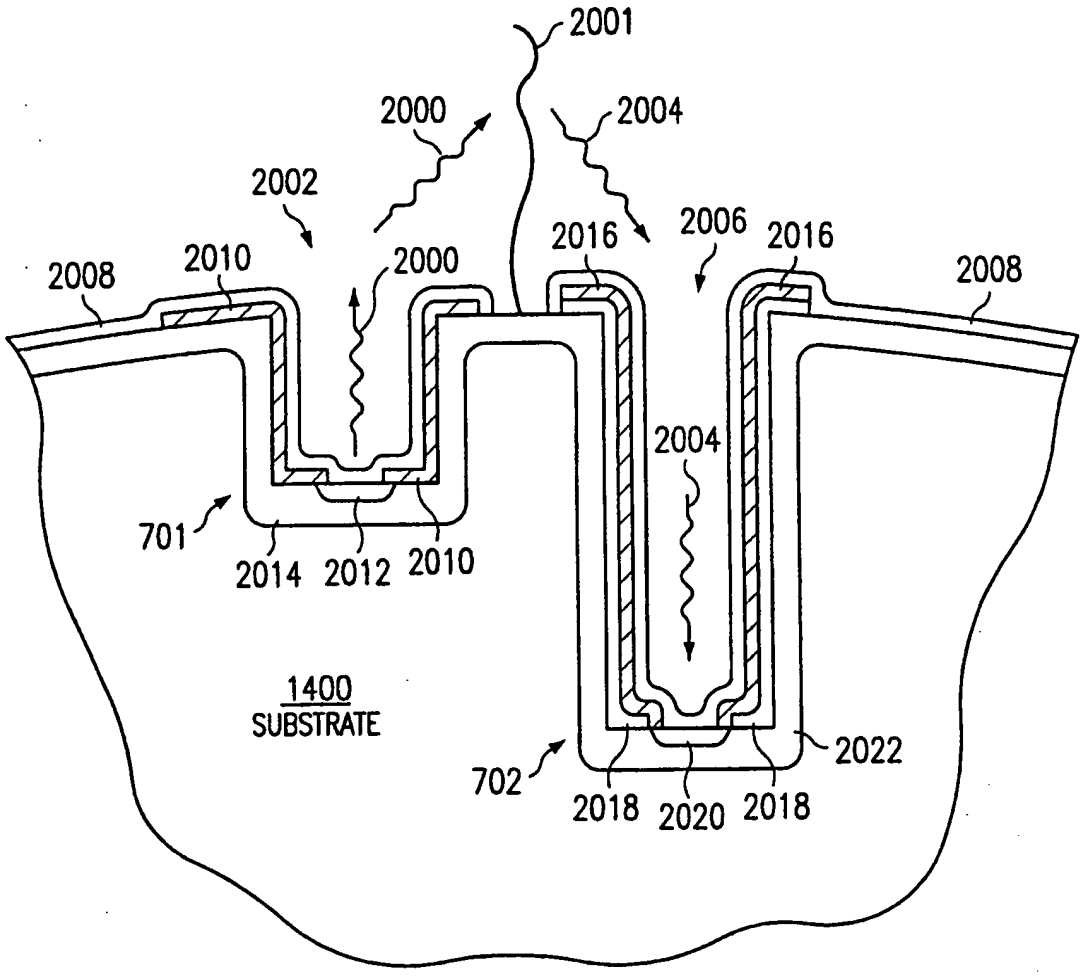

2001
2000
2004
2002
2016
2006
2016
2008
2010
2000
2008
2004
701
2014
2012
2010
1400
SUBSTRATE
2022
702
2018
2020
2018

FIG. 20

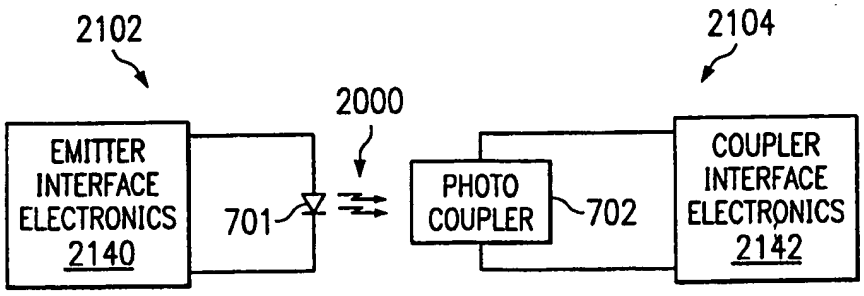
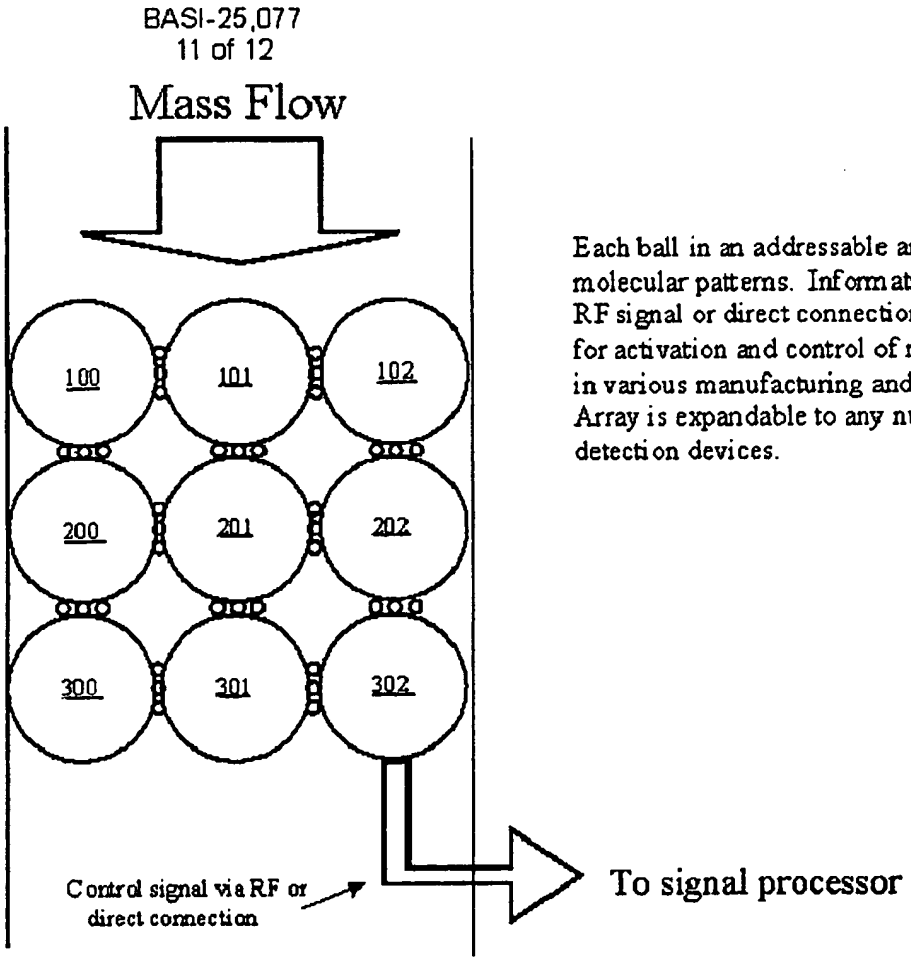

2102
2104
2000
EMITTER
INTERFACE
ELECTRONICS
2140
701
PHOTO
COUPLER
702
COUPLER
INTERFACE
ELECTRONICS
2142

FIG. 21



Each ball in an addressable array senses specific molecular patterns. Information is transferred via RF signal or direct connection to signal processor for activation and control of mass flow distribution in various manufacturing and analytical processes. Array is expandable to any number of molecular detection devices.

FIG.14A

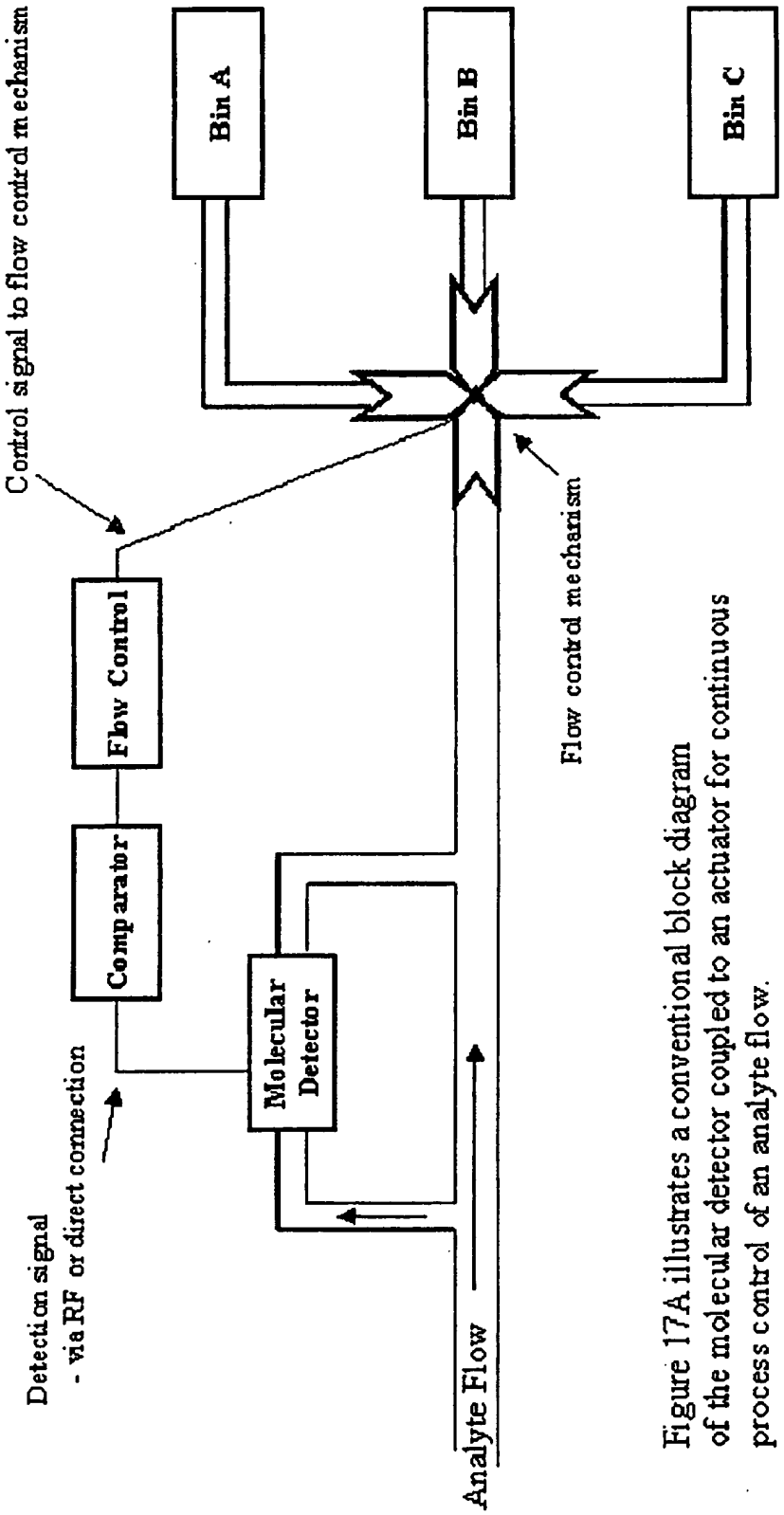


Figure 17A illustrates a conventional block diagram of the molecular detector coupled to an actuator for continuous process control of an analyte flow.

FIG. 17A